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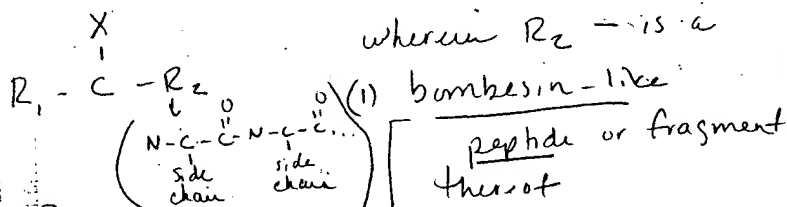
Requestor's Name: Delacort Serial Number: 09/351,057
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Please search the compounds:

X = Oxygen or Sulfur



RECEIVED
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Point of Contact:
Susan Hanley
Technical Info. Specialist
CM1 12C14 Tel: 305-4053

Neuromedin
B or C

or releasing (peptide or protein)
GRP
and

Thanks
please rush
com

(2) wherein R_1 is a
fluorescent moiety

specifically
Bodipy, fluorescein,
FITC or rhodamine

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____ DARC/Questel
____ Other

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(FILE 'HOME' ENTERED AT 10:56:00 ON 16 JUL 2001)

FILE 'HCAPLUS' ENTERED AT 10:56:09 ON 16 JUL 2001

E SLON/AU
 L1 32 S E4-9, E14-17
 L2 1 S BONTER K?/AU
 L3 0 S L1 AND L2
 L4 33 S L1-2
 L5 326968 S FLUORE?
 L6 0 S L4 AND L5
 L7 0 S L4 AND BOMBESIN?
 L8 24 S L4 AND PEPTID?
 L9 3477 S ?BOMBESIN?
 L10 577675 S L5 OR FLUORO?
 L11 93 S L9 AND L10
 L12 34188 S FITC OR RHODAMINE OR FLUORESCIN OR BODIPY
 L13 6 S L11 AND L12
 L14 1696 S GASTRIN-RELEASING
 L15 2616 S L14 OR GRP
 L16 831 S NEUROMEDIN

} no relevant hits from
 inventory search

FILE 'REGISTRY' ENTERED AT 11:02:59 ON 16 JUL 2001

E BOMBESIN/CN
 L17 1 S E3
 E GASTRIN RELEAS?/CN
 E GASTRIN RELEAS?/CN
 E GASTRIN RELEASING/CN
 L18 0 ES E4
 E NEUROMEDIN/CN
 L19 2 S E3-4
 E NEUROMEDIN C/CN
 L20 2 S E3
 E FLUORESCIN/CN
 L21 1 S E3
 L22 1 S FITC/CN
 E RHODAMINE/CN
 L23 58 S E4-96
 L24 1 S BODIPY/CN

L10 = fluorophores, etc ingested
 L26 = specifically named
 fluoro phores
 L25 = Bombesin
 L27 = neuromedin
 L15 = Gastric releasing prot or
 peptide

FILE 'HCAPLUS' ENTERED AT 11:07:58 ON 16 JUL 2001

L25 3531 S L9 OR L17
 L26 36914 S L12 OR L24 OR L23 OR L22 OR L21
 L27 863 S L19-20 OR L16
 L28 10 S (L25 OR L27 OR L15) (L) L26 10 cites
 L29 17 S (L25 OR L27 OR L15) (5A) (L10 OR L26)
 L30 89 S (L25 OR L27 OR L15) (5A) (CONJUGAT? OR MODIFI? OR ATTACH? OR CO
 L31 1 S L29 AND L30
 L32 0 S L31 NOT L28
 L33 5 S L30 AND (L10 OR L26)
 L34 4 S L33 NOT L28
 L35 143 S (L25 OR L27 OR L15) (5A) (COUPL? OR BIOCONJUGAT? OR LINK####)
 L36 1 S L35(P) (L10 OR L26)
 L37 4 S L35 AND (L10 OR L26)
 L38 7 S L34 OR L37
 L39 7 S L38 NOT L28 7 cites
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 E E3+ALL/CT
 L40 825 S E7
 L41 21844 S E2-9
 L42 1 S L40 AND (L25 OR L27 OR L15) 1 cite
 L43 69 S L40(L) (PROTEIN OR ?PEPTID?)
 L44 2 S L43 AND AMIDE 2 cites - type of bond w/ any protein
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 L46 28 S L44-45 NOT (L28 OR L39)
 L47 26 S L46 NOT L44
 L48 15 S L47 AND PY<2000 15 cites - any peptide/prot. conj. to
 L49 87 S (L25 OR L27 OR L15) (S) (PEPTIDE(2A) BOND### OR AMIDE) fluoro phore

} using text
 terms

} using indexing

DELACRIOX 09/351,057

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2 S L49(P) (L10 OR L26)
7 S (L25 OR L27 OR L15) (P) (PEPTIDE(2A)BOND### OR AMIDE) (P) (L10 OR

7cites

=> d bib abs 128 1

L28 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2001 ACS
 AN 2001:475803 HCAPLUS
 TI Serine protease inhibitor causes F-actin redistribution and inhibition of calcium-mediated secretion in pancreatic acini
 AU Singh, Vijay P.; Saluja, Ashok K.; Bhagat, Lakshmi; Hietaranta, Antti J.; Song, Albert; Mykoniatis, Andreas; Van Acker, Gijs J. D.; Steer, Michael L.
 CS Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
 SO Gastroenterology (2001), 120(7), 1818-1827
 CODEN: GASTAB; ISSN: 0016-5085
 PB W. B. Saunders Co.
 DT Journal
 LA English
 AB The present study was undertaken to evaluate the role of serine proteases in regulating digestive enzyme secretion in pancreatic acinar cells. Isolated acini were stimulated by various secretagogues in the presence or absence of cell-permeant serine protease inhibitors 4-(2-aminoethyl)-benzenesulfonyl fluoride and N.alpha.-p-tosyl-L-phenylalanine chloromethyl ketone. F-actin distribution was studied after staining with rhodamine phalloidin. Both cell-permeant serine protease inhibitors blocked amylase secretion in response to secretagogues that use calcium as a second messenger (e.g., caerulein, carbamylcholine, and bombesin) but not to those that use adenosine 3',5'-cyclic monophosphate (cAMP) as a second messenger (e.g., secretin and vasoactive intestinal polypeptide). Incubation of the acini with these inhibitors also resulted in a dramatic redistribution of the F-actin cytoskeleton. This redistribution was energy dependent. Similar redistribution of F-actin from the apical to the basolateral region was also obsd. when acini were incubated with a supramaximally stimulating concn. of caerulein, which is known to inhibit secretion. These results suggest that a serine protease activity is essential for maintaining the normal apical F-actin distribution; its inhibition redistributes F-actin from the apical to the basolateral region and blocks secretion induced by secretagogues that act via calcium. CAMP reverses the F-actin redistribution and hence CAMP-mediated secretion is not affected.

RE.CNT 21
 RE
 (2) Andrews, B; Biochem Biophys Res Commun 2000, V273, P302 HCAPLUS
 (4) Fruman, D; Methods 1996, V9, P146 HCAPLUS
 (5) Harder, K; Biochem J 1994, V298, P395 HCAPLUS
 (6) Kohli, V; Gastroenterology 1999, V116, P168 HCAPLUS
 (7) Martin, B; J Biol Chem 1985, V260, P14932 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 128 2

L28 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:775653 HCAPLUS
 DN 130:151143
 TI Different putative neuromodulators are present in the nerves which distribute to the teleost skeletal muscle
 AU Radaelli, G.; Domeneghini, C.; Arrighi, S.; Mascarello, F.; Veggetti, A.
 CS Department of Animal Sciences, Faculty of Veterinary Medicine, University of Padova, Italy
 SO Histol. Histopathol. (1998), 13(4), 939-947
 CODEN: HHIES; ISSN: 0213-3911
 PB Histology and Histopathology
 DT Journal
 LA English
 AB The presence of putative neuromodulators in the nerve fibers was investigated in white skeletal muscle of 2 teleost fish not taxonomically correlated and showing different patterns of innervation (multiple vs. focal innervation). Cryostat sections of epaxial, hypaxial, and adductor mandibulae (AM) muscles of *Sparus aurata* and *Anguilla anguilla* were stained histochem. for NADPH-diaphorase. Other sections were used for indirect immunohistochem. (streptavidin-biotin and **rhodamine** immunofluorescence methods), employing antibodies specific for putative excitatory or inhibitory peptides, including CGRP, substance P, Met-enkephalin, **bombesin**, and VIP. In addn., ultrastructural observations were performed to describe the morphol. of the motor endplates. A strong immunoreactivity for CGRP and substance P was found in many nerve terminals. Met-enkephalin, **bombesin**, and VIP immunoreactivities were less frequently obsd. No immunoreactivity was obsd. to cholecystokinin, neuropeptide Y, or 5-HT. NADPH-diaphorase was identified in nerve fibers of the AM complex only of *A. anguilla*. Electron microscopy observations evidenced >1 type of synaptic vesicle in motor endplates. Some differences in putative neuromodulator distributions were obsd. in the 2 species and muscle complexes, which may be related to the different taxonomical position as well as the different pattern of innervation of white muscle fibers.

RE.CNT 49

RE

- (1) Andersen, A; Int Rev Cytol 1992, V138, P89 HCAPLUS
- (3) Bell, T; Neurosci Lett 1997, V226, P187 HCAPLUS
- (6) Conlon, J; J Neurochem 1991, V56, P1432 HCAPLUS
- (7) Csillik, B; J Histochem Cytochem 1993, V41, P1547 HCAPLUS
- (8) Dawson, T; Proc Natl Acad Sci USA 1991, V88, P7797 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 128 3

L28 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2001 ACS

AN 1998:617841 HCAPLUS

DN 129:311098

TI A role for the p38 mitogen-activated protein kinase/Hsp 27 pathway in cholecystokinin-induced changes in the actin cytoskeleton in rat pancreatic acini

AU Schafer, Claus; Ross, Sarah E.; Bragado, M. Julia; Groblewski, Guy E.; Ernst, Stephen A.; Williams, John A.

CS Departments of Physiology and Internal Medicine, University of Michigan, Ann Arbor, MI, 48109-0622, USA

SO J. Biol. Chem. (1998), 273(37), 24173-24180

CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB Cholecystokinin (CCK) and other pancreatic secretagogues have recently been shown to activate signaling kinase cascades in pancreatic acinar cells, leading to the activation of extracellular signal-regulated kinases and Jun N-terminal kinases. The authors now show the presence of a third kinase cascade activating p38 mitogen-activated protein (MAP) kinase in isolated rat pancreatic acini. CCK and osmotic stress induced by sorbitol activated p38 MAP kinase within minutes; their effects were dose-dependent, with maximal activation of 2.8- and 4.4-fold, resp. The effects of carbachol and **bombesin** on p38 MAP kinase activity were similar to those of CCK, whereas phorbol ester, epidermal growth factor, and vasoactive intestinal polypeptide stimulated p38 MAP kinase by 2-fold or less. Both CCK and sorbitol also increased the tyrosyl phosphorylation of p38 MAP kinase. Using the specific inhibitor of p38 MAP kinase, SB 203580, the authors found that p38 MAP kinase activity was required for MAP kinase-activated protein kinase-2 activation in pancreatic acini. SB 203580 reduced the level of basal phosphorylation and blocked the increased phosphorylation of Hsp 27 after stimulation with either CCK or sorbitol. CCK treatment induced an initial rapid decrease in total F-actin content of acini, followed by an increase after 40 min. Preincubation with SB 203580 significantly inhibited these changes in F-actin content. Staining of the actin cytoskeleton with **rhodamine**-conjugated phalloidin and anal. by confocal fluorescence microscopy showed disruption of the actin cytoskeleton after 10 and 40 min of CCK stimulation. Pretreatment with SB 203580 reduced these changes. These findings demonstrate that the activation of p38 MAP kinase is involved not only in response to stress, but also in physiol. signaling by gastrointestinal hormones such as CCK, where activation of Gq-coupled receptors stimulates a cascade in which p38 MAP kinase activates MAP kinase-activated protein kinase-2, resulting in Hsp 27 phosphorylation. Activation of p38 MAP kinase, most likely through phosphorylation of Hsp 27, plays a role in the organization of the actin cytoskeleton in pancreatic acini.

=> d bib abs 128 4

L28 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2001 ACS
 AN 1997:633567 HCAPLUS
 DN 127:303487
 TI Bombesin-like peptides stimulate somatostatin release from rat fundic D cells in primary culture
 AU Schaffer, Kirsten; Herrmuth, Hedda; Mueller, James; Coy, David H.; Wong, Helen C.; Walsh, John H.; Classen, Meinhard; Schusdziarra, Volker; Schepp, Wolfgang
 CS Dep. Med. II and Pathology, Technical Univ., Munich, D-81675, Germany
 SO Am. J. Physiol. (1997), 273(3, Pt. 1), G686-G695
 CODEN: AJPHAP; ISSN: 0002-9513
 PB American Physiological Society
 DT Journal
 LA English
 AB In several species, **bombesin**-like neuropeptides stimulate somatostatin release in in vitro preps. of gastric mucosa. We sought to det. if this response is due to a direct effect on fundic D cells. Rat fundic mucosal cells were isolated by pronase E (1% D cells). D cells were sepd. by counterflow elutriation and subsequent d.-gradient centrifugation (Nycodenz) (15% D cells) and grown in primary culture for 48 h (46% D cells). Cultured cells were double stained with affinity-purified rabbit-anti- **gastrin-releasing** peptide (GRP) receptor antibody and mouse monoclonal antibody to human somatostatin. After incubation with **rhodamine**-labeled anti-rabbit and **fluorescein** isothiocyanate-labeled anti-mouse antibodies, reactions were visualized by fluorescence microscopy. All cells pos. for somatostatin had GRP receptors, whereas all non-D cells showed no expression in this G cell-free culture system. Somatostatin release from cultured cells was stimulated by sulfated cholecystokinin octapeptide (CCK-8; EC50 3×10^{-10} M) and epinephrine (EC50 4×10^{-8} M), which are established stimuli for canine fundic D cells. **Bombesin** (EC50 6×10^{-11} M), its mammalian analog GRP-27, and **neuromedin C** (GRP-10) (EC50 1×10^{-10} M, for both) were almost equally potent stimuli of somatostatin release, eliciting maximal response at 10^{-9} M (400-550% above basal). **Neuromedin B** was less potent and effective (maximal response at 10^{-8} M, 230% above basal). [D-Phe6]**bombesin**-(6-13)-OMe, a specific **bombesin** receptor antagonist, inhibited **bombesin**-stimulated somatostatin release in a competitive manner (IC50 9×10^{-8} M). Potentiating interactions were obsd. between **bombesin** and dibutyryl adenosine 3',5'-cyclic monophosphate (dbcAMP) or epinephrine, but not between **bombesin** and CCK-8. We conclude that **bombesin**-like peptides directly stimulate somatostatin release by interacting with specific receptors on rat fundic D cells. **Bombesin**-like peptides appear to induce Ca²⁺-phospholipid-dependent signal-response transduction, as is indirectly suggested by potentiating interactions with dbcAMP or epinephrine.

=> d bib abs 128 5

L28 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2001 ACS

AN 1996:381014 HCAPLUS

DN 125:51226

TI Pasteurella multocida toxin stimulates mitogenesis and cytoskeleton reorganization in Swiss 3T3 fibroblasts

AU Dudet, Laure I.; Chailler, Pierre; Dubreuil, J. Daniel; Martineau-Doize, Beatrice

CS Caculte Medecine Veterinaire, Univ. Montreal, St-Hyacinthe, PQ, J2S 7C6, Can.

SO J. Cell. Physiol. (1996), 168(1), 173-182

CODEN: JCLLAX; ISSN: 0021-9541

DT Journal

LA English

AB In the present study designed to further investigate the effects of Pasteurella multocida toxin (PMT) on cell shape and proliferation, we report that the mitogenic effect of affinity-purified PMT on quiescent 3T3 cells was even superior at 5 ng/mL to that of fetal bovine serum (FBS) or bombesin. This pos. effect was inhibited by heat denaturation and methylamine treatment (this agent blocks internalization). Preincubation of PMT with gangliosides GM1, GM2, or GM3 counteracted its effect on DNA synthesis, suggesting that the toxin binds to GM-type ceramides on target cells. The distribution of F-actin was analyzed in control/treated cells using FITC-conjugated phalloidin. In comparison with FBS and bombesin, PMT triggered a more rapid and profound reorganization of cortical actin into prominent stress fibers after only 5-10 min. This event led to the retraction of cells after only 30 min and ultimately to the induction of mitotic figures. Interestingly, methylamine blocked the effects of PMT on stress fiber formation and cell retraction but not the ruffling response, suggesting that some early events may not require toxin internalization. In summary, these findings indicate that PMT concomitantly exerts a strong mitogenic activity and a rapid stimulation of cytoskeletal rearrangements, possibly after binding to membrane gangliosides and subsequent internalization. We propose that this toxin could be used in the future as a defined inducer of transduction signals involved in cellular proliferation and control of cell shape.

=> d bib abs 128 6

L28 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:298266 HCAPLUS
DN 124:315130
TI Confocal laser scanning microscopy examination of cell distribution in
macroporous microcarriers
AU Bancel, Stephane; Hu, Wei-Shou
CS Department of Chemical Engineering and Materials Science, University of
Minnesota, Minneapolis, MN, 55455-0132, USA
SO Biotechnol. Prog. (1996), 12(3), 398-402
CODEN: BIPRET; ISSN: 8756-7938
DT Journal
LA English
AB Macroporous microcarriers are often used to cultivate animal cells. The
pores in the interior of the beads provide surface and space for cell
growth. It is not clear how anchorage-dependent and suspension cells
populated these microcarriers during cultivation. Confocal laser scanning
microscopy was employed to perform time lapse observation of the cells in
the interior. The structure of the bead was stained with
fluorescein isothiocyanate for visualization, while the cells were
stained with dialkyl indocarbocyanines for tracking over time. It was
obsd. that mouse fibroblastic cells CRE BaG2 did not move extensively
after initial attachment. Some cell divisions were obsd. during the
course of the expts., and essentially all cells remained viable
throughout. Few hybridoma cells were deposited into the pores in the
interior of the microcarriers. The results suggest that the occupancy of
the internal vol. by cells after prolonged cultivation is largely due to
the growth of cells that are deposited in the interior as opposed to the
migration of cells from the external surface into the interior. This
method of observing cell behavior in a three-dimensional structure may
find applications in other three-dimensional cell culture systems. The
animation of time lapse sections is available on the worldwide web at
http://www.cems.umn.edu/.apprx.wshu_grp/acre/microcarrier.html.

=> d bib abs 128 7

L28 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2001 ACS
 AN 1995:660294 HCAPLUS
 DN 123:314467
 TI Modification of (Lys3)-**bombesin** with **fluorescein**
 5'-isothiocyanate
 AU Alberto, Mazzini; Cristina, Ruggi; Roberta, Bedotti; Paolo, Cavatorta;
 Roberto, Favilla
 CS Department of Physics, University of Parma, Parma, 43100, Italy
 SO Med., Biol., Environ. (1993), 21(2), 619-28
 CODEN: MBENDX; ISSN: 0302-0800
 DT Journal
 LA English
 AB **Fluorescein** 5'-isothiocyanate (**FITC**) was used to
 modify the lysine residue of (Lys3)-**bombesin**, a fully active
 analog of the hormone **bombesin**. The incorporation of
FITC was performed at alk. pH, where the stability of **FITC**
 and (Lys3)-**bombesin** were previously analyzed. The fluorescent
 peptide was purified chromatog. on a hydrophobic interaction column and
 spectroscopically characterized. Preliminary expts. on proliferation of
 cultured Swiss 3T3 cells indicated that **FITC** labeling does not
 alter the biol. activity of the modified hormone.

=> d bib abs 128 8

L28 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2001 ACS.
 AN 1995:410215 HCAPLUS
 DN 122:178902
 TI Direct observation of endocytosis of gastrin releasing peptide and its receptors
 AU Grady, Eileen F.; Slice, Lee W.; Brant, William O.; Walsh, John H.; Payan, Donald G.; Bunnett, Nigel W.
 CS Departments of Surgery, Physiology and Medicine, Univ. of California, San Francisco, CA, 94143, USA
 SO J. Biol. Chem. (1995), 270(9), 4603-11
 CODEN: JBCHA3; ISSN: 0021-9258
 DT Journal
 LA English
 AB Endocytosis of the **gastrin releasing peptide receptor (GRP-R)** may regulate cellular responses to **GRP**. The authors obsd. endocytosis in transfected epithelial cells by confocal microscopy using cyanine 3-**GRP** (cyanine 3.18-labeled **gastrin releasing peptide**) and **GRP-R** antibodies. At 4.degree., cy3-**GRP** and **GRP-R** were confined to the plasma membrane. After 5 min at 37.degree., ligand and receptor were internalized into early endosomes with **fluorescein isothiocyanate-transferrin**. After 10 min, cy3-**GRP** and **GRP-R** were in perinuclear vesicles, and at 60 min cy3-**GRP** was in large, central vesicles, while **GRP-R** was at the cell surface. The authors quantified surface **GRP-R** using an antibody to an extracellular epitope and an 125I-labeled secondary antibody. After exposure to **GRP**, there was a loss and subsequent recovery of surface **GRP-R**. Recovery was unaffected by cycloheximide, and thus independent of new protein synthesis, but was attenuated by acidotropic agents, and therefore required endosomal acidification. Internalization of 125I-**GRP**, assessed using an acid wash, was maximal after 10-20 min, and was clathrin-mediated since it was inhibited by hyperosmolar sucrose and phenylarsine oxide. Thus, **GRP** and its receptor are rapidly internalized into early endosomes and then dissoc. in an acidified compartment. **GRP** is probably degraded whereas the **GRP-R** recycles.

=> d bib abs 128 9

L28 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2001 ACS

AN 1994:601751 HCAPLUS

DN 121:201751

TI Agonist-induced rise in intracellular calcium of lens epithelial cells: effects on the actin cytoskeleton

AU Rafferty, Nancy S.; Rafferty, Keen A.; Ito, Etsuro

CS Northwestern University, Chicago, IL, USA

SO Exp. Eye Res. (1994), 59(2), 191-201

CODEN: EXERA6; ISSN: 0014-4835

DT Journal

LA English

AB Primary cultures of rabbit and skate lens epithelia were used to investigate the effect of calcium release from intracellular stores upon the actin cytoskeleton. Primary cultures were loaded with fura-2 AM and intracellular calcium, i.e., $(Ca^{2+})_i$, was quantitated using a Hamamatsu Photonics digital imaging system. Agonists used were **bombesin**, inositol-1,4,5-trisphosphate (IP3), thapsigargin (Tg), neuropeptide Y (NPY) and calcium chloride. Recordings were typically made on seven cells in each case. The authors found that IP3 caused a 6-fold immediate release of $(Ca^{2+})_i$ in rabbit cells, but skate cells showed no response unless permeabilized with saponin, whereupon an increase of about 50% occurred. Tg induced release from internal stores in rabbit cells, but had no effect on skate cells. **Bombesin** caused a large increase in $(Ca^{2+})_i$ release in both, while NPY had no effect in either. Skate cells incubated in calcium-free EGTA-Ringer's soln. responded rapidly to addn. of 5 mM $CaCl_2$, whereas only three of 35 rabbit cells responded, and in gradual fashion. After calcium imaging, the cells were fixed and stained with **rhodamine** phalloidin or with an antibody against IP3 receptor (IP3R) conjugated to **FITC**. Fluorescence microscopy revealed that the actin cytoskeleton had reorganized from the normal stress fiber pattern into polygonal networks. Tg caused the same structures to form in rabbit cells, but **bombesin** had no effect. IP3 receptor was located intracellularly, presumably on endoplasmic reticulum, and was not assocd. with plasma membranes. The rapid response of rabbit cells may have been caused by the DMSO in which fura-2 was dissolved. The authors have found an interesting difference in agonist-induced calcium release between rabbit and skate cells. The latter may utilize either a Ca-Na exchanger or capacitative calcium entry, which could reflect a difference in lens accommodative mechanisms. This seems relevant in view of the fact that the rabbit lens accommodates through change in shape, whereas the skate lens does so through translation of position.

=> d bib abs 128 10

L28 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1991:401318 HCAPLUS
DN 115:1318
TI Biotinylation of a bombesin/gastrin-releasing peptide analog for use as a receptor probe
AU Anton, Peter A.; Reeve, Joseph R., Jr.; Rivier, Jean E.; Vidrich, Alda; Schepp, Wolfgang; Shanahan, Fergus
CS Dep. Med., UCLA, Los Angeles, CA, 90024, USA
SO Peptides (Fayetteville, N. Y.) (1991), 12(2), 375-81
CODEN: PPTDD5; ISSN: 0196-9781
DT Journal
LA English
AB The development of a biotinylated **bombesin-gastrin-releasing** peptide (GRP) for use as a receptor probe is reported. The lysine13 of a GRP-27 was substituted by arginine and lysine was added to the amino terminus. Biotinylation of the N-terminal lysine was performed. The biotinylated peptide was purified by HPLC and characterized by mass spectral anal. Binding studies with murine Swiss 3T3 fibroblasts, cells known to express **bombesin/GRP** receptors, yielded a dissocn. curve for the biotinylated GRP-27 analog (biotin-Lysyl[Asp12,Arg13]GRP-27) which was nearly identical to that of native GRP. Using studies of gastrin release from isolated canine G cells, equipotent functional activity of the biotinylated probe and unmodified GRP was demonstrated. Measurements of retained 125I-avidin confirmed that the biotin/avidin interaction could occur once the biotin-peptide complex was bound. Applicability of the probe was demonstrated with fluorescent microscopy using avidin-FITC on Swiss 3T3 fibroblasts. In conclusion, a novel biotinylated **bombesin/GRP** analog has been developed which retains the functional characteristics of the native peptide and is a useful probe for receptor studies.

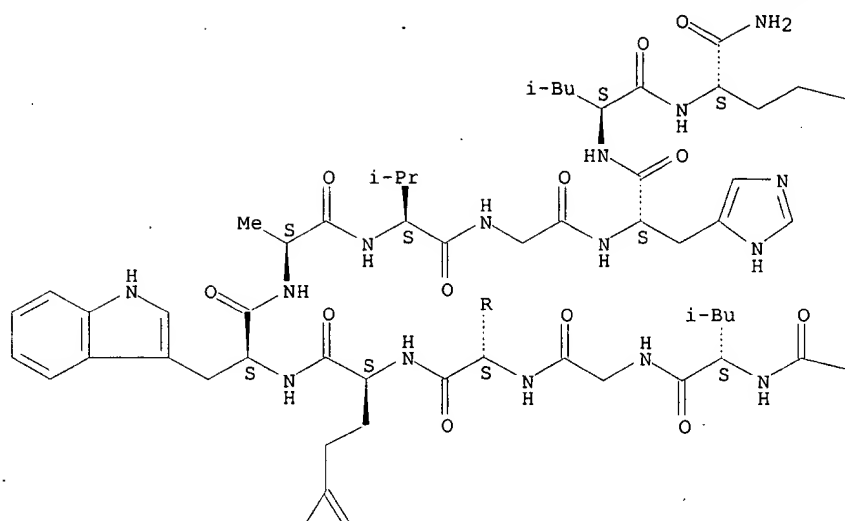
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L39 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 2000:878948 HCAPLUS
 DN 134:161051
 TI G protein-coupled receptor signaling in human ductal pancreatic cancer cells: neurotensin responsiveness and mitogenic stimulation
 AU Ryder, Nova M.; Guha, Sushovan; Hines, Oscar J.; Reber, Howard A.; Rozengurt, Enrique
 CS Department of Surgery School of Medicine, University of California, Los Angeles, CA, 90095-1786, USA
 SO J. Cell. Physiol. (2001), 186(1), 53-64
 CODEN: JCLLAX; ISSN: 0021-9541
 PB Wiley-Liss, Inc.
 DT Journal
 LA English
 AB Neuropeptides and their corresponding G protein-coupled receptors (GPCRs) are increasingly implicated in the autocrine/paracrine stimulation of growth of human cancers. We report that neurotensin induced rapid Ca²⁺ mobilization from intracellular stores followed by Ca²⁺ influx in five human ductal pancreatic cancer cell lines: HPAF-II, Capan-1, Capan-2, PANC-1, and MIA PaCa-2. In addn., most cell lines exhibited Ca²⁺ responses to multiple neuropeptides including bombesin, bradykinin, cholecystokinin, and vasopressin and to bioactive lipids, including lysophosphatidic acid (LPA), that also act via GPCRs. The well-differentiated line HPAF-II responded to at least seven independent GPCR agonists. The concns. of neurotensin required to induce half-maximal effects (EC₅₀) in HPAF-II and PANC-1 cells were 5 and 8nM, resp. Digital fluorescence image anal. to measure Ca²⁺ responses in single cells revealed that 90% or more of HPAF-II and PANC-1 cells responded to 10nM neurotensin. Addn. of neurotensin to PANC-1 cells also induced rapid and dose-dependent extracellular-regulated protein kinase (ERK-1 and ERK-2) activation and subsequently, stimulated DNA synthesis. The signaling complexity of GPCRs uncovered by these studies reveals a new aspect in the biol. of human pancreatic cancer and could offer the basis for new approaches to the treatment of this disease.

IT 31362-50-2, Bombesin
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (G protein-coupled receptor signaling in human ductal pancreatic cancer cells: neurotensin responsiveness and mitogenic stimulation)
 RN 31362-50-2 HCAPLUS
 CN Bombesin (9CI) (CA INDEX NAME)

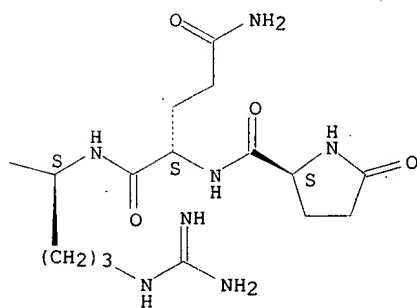
Absolute stereochemistry.

PAGE 1-A

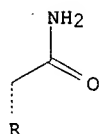
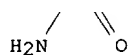


PAGE 1-B

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PAGE 2-A



RE.CNT 47

RE

- (1) Alessi, D; J Biol Chem 1995, V270, P27489 HCAPLUS
 - (2) Avis, I; Mol Carcinog 1993, V8, P214 HCAPLUS
 - (4) Castagliuolo, I; J Clin Invest 1999, V103, P843 HCAPLUS
 - (5) Detjen, K; Gastroenterology 1997, V112, P952 HCAPLUS
 - (6) Elek, J; Anticancer Res 2000, V20, P53 HCAPLUS
- ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 2

L39 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS

AN 2000:842014 HCAPLUS

DN 134:21520

TI Novel cyanine and indocyanine dye bioconjugates for biomedical applications

IN Achilefu, Samuel; Dorshow, Richard Bradley; Bugaj, Joseph Edward; Rajagopalan, Raghavan

PA Mallinckrodt Inc., USA

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000071162	A2	20001130	WO 2000-US11060	20000426
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6217848	B1	20010417	US 1999-325769	19990604
PRAI	US 1999-135060	P	19990520		
	US 1999-325769	A	19990604		

OS MARPAT 134:21520

AB Dye-peptide conjugates useful for diagnostic imaging and therapy are disclosed. The dye-peptide conjugates include several cyanine dyes with a variety of bis- and tetrakis(carboxylic acid) homologs. The small size of the compds. allows more favorable delivery to tumor cells as compared to larger mol. wt. imaging agents. The various dyes are useful over the range of 350-1300 nm, the exact range being dependent upon the particular dye. Use of dimethylsulfoxide helps to maintain the **fluorescence** of the compds. The mols. of the invention are useful for diagnostic imaging and therapy, in endoscopic applications for the detection of tumors and other abnormalities and for localized therapy, for photoacoustic tumor imaging, detection and therapy, and for sonofluorescence tumor imaging, detection and therapy. For example, monooctreotate-bisethylcarboxymethyl indocyanine dye (Cytate 1) was prepd. (yield of 80%) and evaluated in the CA20948 Lewis rat model of pancreatic acinar carcinoma. Using the CCD camera, strong localization of this dye was obsd. in the tumor at 90 min post injection. At 19 h post injection the animal was again imaged and tumor visualization was easily obsd. showing specificity of this agent for somatostatin receptors present in this tumor line.

IT 31362-50-2DP, Bombesin, conjugates with cyanine dyes

RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)

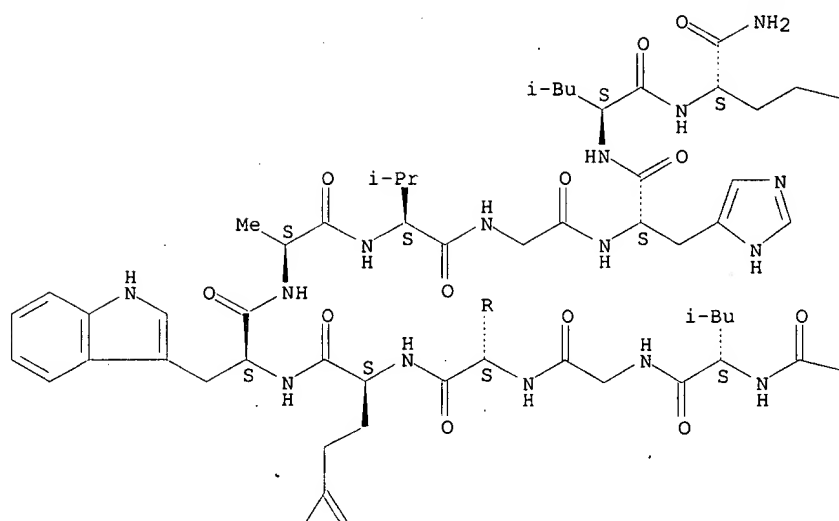
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

RN 31362-50-2 HCAPLUS

CN Bombesin (9CI) (CA INDEX NAME)

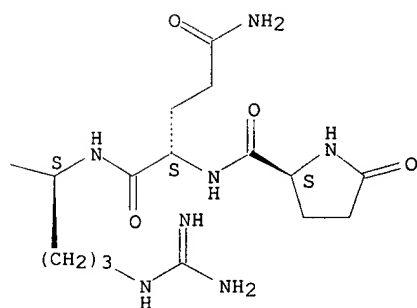
Absolute stereochemistry.

PAGE 1-A

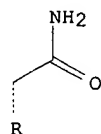
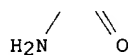


PAGE 1-B

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PAGE 2-A



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L39 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 IC ICM A61K041-00
 CC 63-8 (Pharmaceuticals)
 Section cross-reference(s): 1, 8, 14, 34, 41
 ST cyanine dye peptide conjugate imaging diagnosis therapy; tumor imaging
 therapy cyanine dye peptide conjugate
 IT Surgery
 (LAGS (laser-assisted guided surgery); bioconjugates of cyanine and
 indocyanine dyes with peptides for diagnostic imaging and therapy)
 IT Pancreas, neoplasm
 (acinar cell, adenocarcinoma; bioconjugates of cyanine and indocyanine
 dyes with peptides for diagnostic imaging and therapy)
 IT Diagnosis
 (agents; bioconjugates of cyanine and indocyanine dyes with peptides
 for diagnostic imaging and therapy)
 IT Antiarteriosclerotics
 (antiatherosclerotics; bioconjugates of cyanine and indocyanine dyes
 with peptides for diagnostic imaging and therapy)
 IT Antitumor agents
 Atherosclerosis
 Cyanine dyes
 Drug delivery systems
 Fluorescence
 Imaging agents
 Light scattering
 Optical absorption
 Photodynamic therapy
 Thrombus
 (bioconjugates of cyanine and indocyanine dyes with peptides for
 diagnostic imaging and therapy)
 IT Somatostatin receptors
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (bioconjugates of cyanine and indocyanine dyes with peptides for
 diagnostic imaging and therapy)
 IT Prostate gland
 (carcinoma; bioconjugates of cyanine and indocyanine dyes with peptides
 for diagnostic imaging and therapy)
 IT Glycopeptides
 RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological
 study); PREP (Preparation); USES (Uses)
 (conjugates with cyanine dyes; bioconjugates of cyanine and indocyanine
 dyes with peptides for diagnostic imaging and therapy)
 IT Peptidomimetics
 (conjugates, with cyanine dyes; bioconjugates of cyanine and
 indocyanine dyes with peptides for diagnostic imaging and therapy)
 IT Antibodies
 Hormones, animal, biological studies
 Oligosaccharides, biological studies
 Peptides, biological studies
 RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological
 study); PREP (Preparation); USES (Uses)
 (conjugates, with cyanine dyes; bioconjugates of cyanine and
 indocyanine dyes with peptides for diagnostic imaging and therapy)
 IT Tomography
 (contrast agents; bioconjugates of cyanine and indocyanine dyes with
 peptides for diagnostic imaging and therapy)
 IT Imaging agents
 (contrast, tomog.; bioconjugates of cyanine and indocyanine dyes with
 peptides for diagnostic imaging and therapy)
 IT Pancreas, neoplasm
 (duct cell adenocarcinoma; bioconjugates of cyanine and indocyanine
 dyes with peptides for diagnostic imaging and therapy)
 IT Imaging
 (**fluorescent**, endoscopic; bioconjugates of cyanine and
 indocyanine dyes with peptides for diagnostic imaging and therapy)
 IT Photoacoustic effect
 (imaging; bioconjugates of cyanine and indocyanine dyes with peptides

- for diagnostic imaging and therapy)
- IT Neoplasm
(metastasis, micro-; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT Imaging
(photoacoustic; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT Sonoluminescence
(sonofluorescence; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT 302794-43-ODP, conjugates with cyanine dyes
RL: BPR (Biological process); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT 64-17-5, Ethanol, biological studies 67-68-5, Dimethylsulfoxide, biological studies
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT 25679-24-7P 83150-76-9P, Octreotide 105466-87-3P 195825-84-4P 302794-43-OP 309916-88-9P 309916-89-OP 309916-90-3P
RL: PNU (Preparation, unclassified); RCT (Reactant); PREP (Preparation)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT 590-92-1, 3-Bromopropanoic acid 4224-70-8, 6-Bromohexanoic acid 41532-84-7, 1,1,2-Trimethyl-[1H]-benz[e]indole 309916-91-4 309916-92-5
RL: RCT (Reactant)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
- IT 9011-97-6DP, Cholecystokinin, conjugates with cyanine dyes
31362-50-2DP, Bombesin, conjugates with cyanine dyes 37221-79-7DP, Vasoactive intestinal peptide, conjugates with cyanine dyes 39379-15-2DP, Neurotensin, conjugates with cyanine dyes 51110-01-1DP, Somatostatin, conjugates with cyanine dyes 83150-76-9DP, Octreotide, conjugates with cyanine dyes
RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

=> d bib abs hitstr 3

L39 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 2000:84648 HCAPLUS
 DN 132:141941
 TI Conjugates and fusion proteins for treating secondary tissue damage and other inflammatory conditions and disorders
 IN McDonald, John R.; Coggins, Philip J.
 PA Osprey Pharmaceuticals Limited, Can.
 SO PCT Int. Appl., 204 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000004926	A2	20000203	WO 1999-CA659	19990721
	WO 2000004926	A3	20001102		
	W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, SD, SI, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
	AU 9948918	A1	20000214	AU 1999-48918	19990721
	EP 1098664	A2	20010516	EP 1999-932572	19990721
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
PRAI	US 1998-120523	A2	19980722		
	WO 1999-CA659	W	19990721		
AB	Conjugates contg. as a ligand a chemokine receptor-targeting agent, such as chemokines, and a targeted agent, such as a toxin are provided. These conjugates are used to treat inflammatory responses assocd. with activation, proliferation and migration of immune effector cells, including leukocyte cell types, neutrophils, macrophages, and eosinophils. The conjugates provided herein are used to lessen or inhibit these processes to prevent or at least lessen the resulting secondary effects. In particular, the conjugates are used to target toxins to receptors on secondary tissue damage-promoting cells. The ligand moiety can be selected to deliver the cell toxin to such secondary tissue damage-promoting cells as mononuclear phagocytes, leukocytes, natural killer cells, dendritic cells, and T and B lymphocytes, thereby suppressing the proliferation, migration, or physiol. activity of such cells. Among preferred conjugates are fusion proteins having a chemokine, or a biol. active fragment thereof, as the ligand moiety linked to a cell toxin via a peptide linker of from 2 to about 60 amino acid residues.				

=> d bib abs hitstr 4

L39 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1999:427643 HCAPLUS
 DN 131:318406
 TI A comparative linkage and physical map of bovine chromosome 24 with human chromosome 18
 AU Larsen, Niels J.; Hayes, Helen; Bishop, Michael; Davis, Scott K.; Taylor, Jeremy F.; Kirkpatrick, Brian W.
 CS Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI, 53706, USA
 SO Mamm. Genome (1999), 10(5), 482-487
 CODEN: MAMGEC; ISSN: 0938-8990
 PB Springer-Verlag New York Inc.
 DT Journal
 LA English
 AB Polymorphic microsatellites have been developed in the vicinity of nine genes on bovine chromosome (BTA) 24, all orthologous to genes on human chromosome (HSA) 18. The microsatellites have been isolated from bacterial and yeast artificial chromosome clones contg. the genes. A linkage map was developed including these polymorphic markers and four anonymous, published microsatellites. Yeast artificial chromosomes contg. six of these genes have also been mapped using **fluorescent** in situ hybridization (FISH), thereby tying the linkage map together with the phys. map of BTA24. Comparing gene location on HSA18 and BTA24 identifies four regions of conserved gene order, each contg. at least two genes. These genes identify six regions of conserved order between human and mouse, two more than in the human-bovine comparison. The breakpoints between regions of conserved order for human-bovine are also breakpoints in the human-mouse comparison. The centromere identifies a fifth conserved region if the BTA24 centromere is orthologous with the HSA18 centromere.
 RE.CNT 39
 RE
 (1) Agaba, M; Mammalian Genome 1997, V8, P530 HCAPLUS
 (2) Ballabio, A; Nature Genetics 1993, V3, P277 HCAPLUS
 (3) Barendse, W; Mammalian Genome 1997, V8, P21 HCAPLUS
 (5) Brown, J; Molecular and Cellular Probes 1995, V9, P53 HCAPLUS
 (6) Brownstein, M; BioTechniques 1996, V20, P1004 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 5

L39 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2001 ACS

AN 1991:651669 HCAPLUS

DN 115:251669

TI A method for the stepwise, controlled synthesis of chemical species, particularly peptides, on protein substrates, coupled products obtained by the method, and the use of these coupled products, e.g. as vaccines

IN Houen, Gunnar; Holm, Arne

PA Den.

SO PCT Int. Appl., 106 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9108220	A1	19910613	WO 1990-DK311	19901130/
W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, GR, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU, US				
RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				
AU 9168929	A1	19910626	AU 1991-68929	19901130
PRAI DK 1989-6085		19891201		
WO 1990-DK311		19901130		

AB Chem. species, esp. peptides, are synthesized by a stepwise, controlled process using a proteinaceous substances as the synthesis substrate. The coupled products obtained by the process can be used, e.g., as vaccines, matrix materials, or carrier mols. The products, including peptides and peptide derivs., prep'd. by the method are also claimed. Bovine serum albumin (BSA) was placed in a silylated reaction vessel and the CO₂H groups were diethylamidated before coupling glutamic acid as the Fmoc (9-fluorenylmethyloxycarbonyl) and tert-Bu protected Dhbt (3-hydroxy-3,4-dihydrobenzotriazin-4-one ester, blocking remaining amino groups with acetic anhydride, and sequentially coupling Fmoc- and side chain-protected Dhbt esters of lysine, serine, threonine, aspartic acid, methionine, and serine. Piperidine was used to remove the Fmoc protecting group between couplings. Side chain protection groups were removed in CH₂Cl₂/F₃CCO₂H (1:1 vol./vol.) at 0.degree.. The product had an av. of 35 synthesized peptide chains per BSA mol. The coupled product was used to raise antibodies to Ser-Met-Asp-Thr-Ser-Lys-Glu in rabbits.

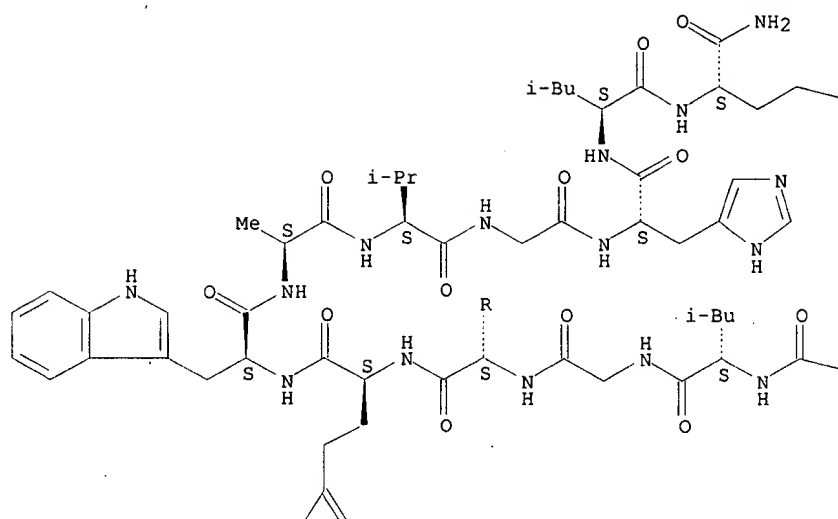
IT 31362-50-2D, Bombesin, conjugates with protein carrier 102577-19-5D, Neuromedin B, conjugates with protein carrier 102577-22-0D, Neuromedin C, conjugates with protein carrier
 RL: RCT (Reactant)
 (stepwise synthesis of, for vaccines and other uses)

RN 31362-50-2 HCAPLUS

CN Bombesin (9CI) (CA INDEX NAME)

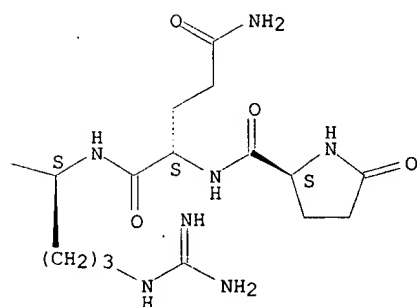
Absolute stereochemistry.

PAGE 1-A

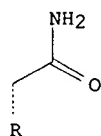
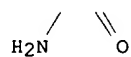


PAGE 1-B

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RN 102577-19-5 HCAPLUS
CN Neuromedin B (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

RN 102577-22-0 HCAPLUS
CN Neuromedin C (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

=> d bib abs hitstr 6

L39 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1991:400778 HCAPLUS
 DN 115:778
 TI Covalently-linked complexes and methods for enhanced cytotoxicity and imaging
 IN Anderson, David C.; Morgan, A. Charles; Abrams, Paul G.; Nichols, Everett J.; Fritzberg, Alan R.
 PA NeoRx Corp., USA
 SO Eur. Pat. Appl., 23 pp.
 CODEN: EPXXDW
 DT Patent
 LA English
 FAN.CNT 1

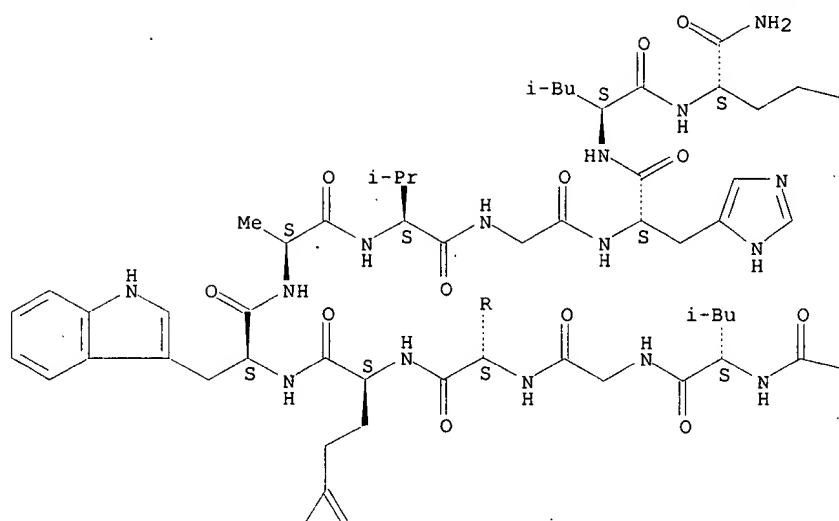
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PI	EP 359347	A2	19900321	EP 1989-250014	19890814
	EP 359347	A3	19900418		
	EP 359347	B1	19921223		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	US 5135736	A	19920804	US 1988-232337	19880815
	US 5169933	A	19921208	US 1989-390241	19890807
	CA 1334513	A1	19950221	CA 1989-608198	19890811
	JP 02124833	A2	19900514	JP 1989-209992	19890814
	AT 83669	E	19930115	AT 1989-250014	19890814
PRAI	US 1988-232337		19880815		
	EP 1989-250014		19890814		

AB Covalently-linked complexes (CLCs) for targeting a defined population of cells comprise a targeting protein (e.g. antibody, hormone, enzyme, etc.), a cytotoxic agent (e.g. radionuclide, toxin, drug, etc.) an enhancing moiety capable of enhancing CLC-target cell interaction (e.g. a translocating/internalizing moiety, an anchoring peptide, membrane-sol. hydrophobic mol., etc.). The CLCs are used to enhance in vivo cytotoxicity and imaging (no data). Translocating peptide, Cys-Gly-Glu-Ala-Ala-Leu-Ala(Glu-Ala-Leu-Ala)4-Glu-Ala-Leu-Glu-Ala-Leu-Ala-Ala-NH₂, is conjugated via succinimidyl 4(N-maleimidemethyl)cyclohexane-1-carboxylate (SMCC) to reduced toxin A chain. The conjugate is reacted with iminothiolane to generate further thiol groups which are then bonded to reduced antibody to prep. translocating peptide-ricin A chain-antibody CLC.

IT 31362-50-2D, Bombesin, conjugates with cytotoxic agent and targeting protein
 RL: BIOL (Biological study)
 (cell targeting with, for enhanced cytotoxicity and imaging)
 RN 31362-50-2 HCAPLUS
 CN Bombesin (9CI) (CA INDEX NAME)

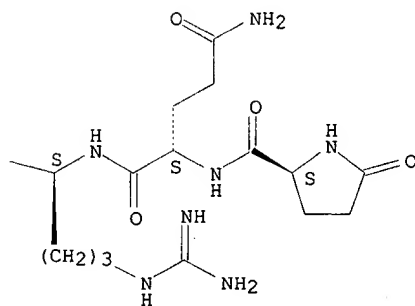
Absolute stereochemistry.

PAGE 1-A

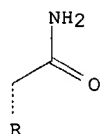
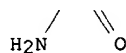


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PAGE 2-A



DELACRIOX 09/351,057

=> d bib abs hitstr 7

L39 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2001 ACS

AN 1988:564243 HCAPLUS

DN 109:164243

TI Pathway of phospholipase C activation initiated with platelet-derived growth factor is different from that initiated with vasopressin and bombesin

AU Hasegawa-Sasaki, Hiroko; Lutz, Frieder; Sasaki, Terukatsu

CS Dep. Biochem., Sapporo Med. Coll., Sapporo, 060, Japan

SO J. Biol. Chem. (1988), 263(26), 12970-6

CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB The effects of GTP, guanosine 5'-O-(2-thiodiphosphate) (GDP.beta.S), and guanosine 5'-O-(3-thiotriphosphate) (GTP.gamma.S) on polyphosphoinositide hydrolysis stimulated by growth factors were studied in rat fibroblast WFB cells made permeable to nucleotides by treatment with either saponin or *Pseudomonas aeruginosa* cytotoxin. Platelet-derived growth factor (PDGF), vasopressin, and bombesin elicited inositol phosphate prodn. in the permeabilized WFB cells in the absence of added GTP. GDP.beta.S, a competitive inhibitor of GTP-binding proteins (G-proteins), markedly reduced the bombesin- and vasopressin-stimulated prodn. of inositol phosphates. However, the PDGF-stimulated prodn. of inositol phosphates was not affected by the addn. of GDP.beta.S. GTP.gamma.S, an agonist of G-proteins, largely enhanced the vasopressin- and bombesin-stimulated hydrolysis of inositol lipids when added at 10-100 .mu.M. In the presence of GTP.gamma.S, the PDGF-stimulated hydrolysis of inositol lipids was not enhanced, but was reduced: 100 .mu.M GTP.gamma.S reduced the stimulated hydrolysis to about a half of the control level. Only GTP.gamma.S and no other nucleoside triphosphates, had these effects. Activation of G-proteins in WFB cells by **fluoroaluminate** resulted in the inhibition of inositol phosphate prodn. elicited with not only PDGF, but also with vasopressin and bombesin. Thus, a G-protein **couples** vasopressin and **bombesin** receptors to the activation of phospholipase C. Coupling of the PDGF receptor to phospholipase C is not mediated through a G-protein. The results obtained by the use of GTP.gamma.S and NaF plus AlCl3 suggest competition for a phospholipase C between an inhibitory G-protein and a transducer protein constituting the PDGF receptor-linked pathway of phospholipase C activation.

DELACRIOX 09/351,057

=> d bib abs hitstr 8

7 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
The answer numbers requested are not in the answer set.
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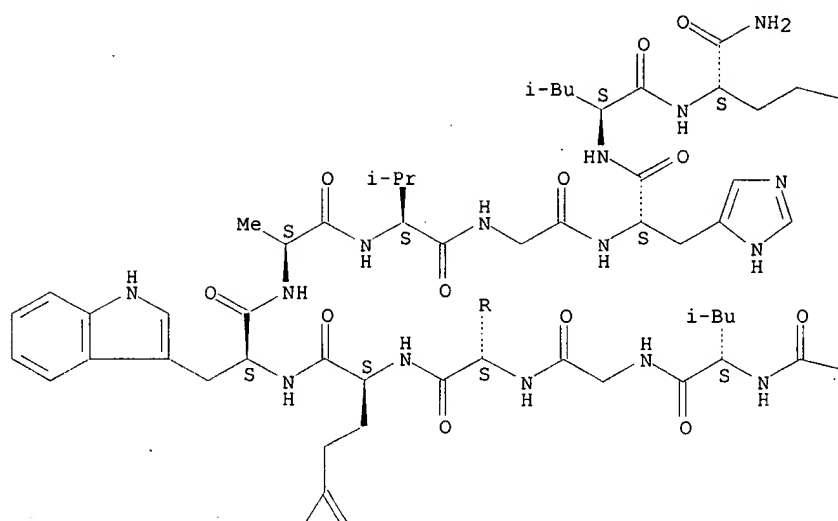
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L42 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2001 ACS
 AN 1999:181069 HCAPLUS
 DN 130:333120
 TI Visualization of internalization and recycling of the **gastrin releasing** peptide receptor-green fluorescent protein chimera expressed in epithelial cells
 AU Slice, Lee W.; Yee, Hal F., Jr.; Walsh, John H.
 CS CURE: VA/UCLA Gastroenteric Biology Center and the Department of Medicine, Division of Digestive Diseases, University of California, Los Angeles, CA, 90095, USA
 SO Recept. Channels (1998), 6(3), 201-212, 5 plates
 CODEN: RCHAE4; ISSN: 1060-6823
 PB Harwood Academic Publishers
 DT Journal
 LA English
 AB **Gastrin releasing peptide (GRP)** regulates crit. gastrointestinal functions via the **GRP** receptor (GRPR). GRPR internalization and recycling have been proposed to play an important role in the cellular response to **GRP**. Our aim was to develop a direct method for investigating GRPR trafficking in living cells. A chimeric protein, consisting of GRPR fused to green fluorescent protein (GFP), was expressed in epithelial cells. Ligand and receptor interactions were examd. with radiolabeled agonist and fluorescent imaging. In comparison with epithelial cells expressing wild-type GRPR, the GRPR-GFP expressing cells showed similar ligand binding affinity, **GRP**-stimulated Ca²⁺ signaling, and **GRP**-initiated internalization. In GRPR-GFP expressing cells treated with fluorescently labeled ligand, receptor and ligand trafficking was directly visualized. Upon ligand binding, the receptor-ligand complex coalesced into vesicles prior to internalization and migration to the perinuclear space. Whereas a portion of the receptors were obsd. to return to the plasma membrane, the ligand remained in the perinuclear space. Hyperosmolar soln. prevented ligand and receptor internalization, and bafilomycin inhibited receptor recycling. We demonstrate that GRPR-GFP is physiol. similar to wild-type GRPR, and permits direct visualization of intracellular trafficking processes in individual living cells with minimal toxicity over several hours.
 IT 31362-50-2, **Bombesin**
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (visualization of internalization and recycling of **gastrin releasing** peptide receptor-green fluorescent protein chimera expressed in epithelial cells)
 RN 31362-50-2 HCAPLUS
 CN Bombesin (9CI) (CA INDEX NAME)

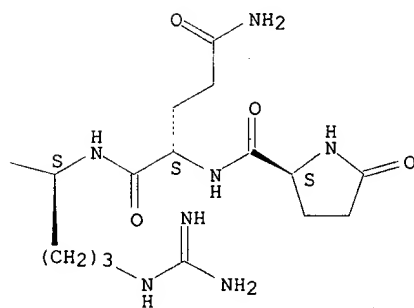
Absolute stereochemistry.

PAGE 1-A

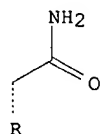
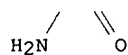


PAGE 1-B

SMe



PAGE 2-A



RE.CNT 21

RE

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 - (2) Bowman, E; Proc Natl Acad Sci USA 1988, V85, P7972 HCAPLUS
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- ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 144 1

L44 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2001 ACS

AN 1999:168147 HCAPLUS

DN 130:296981

TI Fluorescent Pseudo-Peptide Linear Vasopressin Antagonists: Design, Synthesis, and Applications

AU Durroux, Thierry; Peter, Marion; Turcatti, Gerardo; Chollet, Andre; Balestre, Marie-Noeelle; Barberis, Claude; Seyer, Rene

CS INSERM U 469 and CNRS UPR 9023, CCIPE, Montpellier, 34094, Fr.

SO J. Med. Chem. (1999), 42(7), 1312-1319

CODEN: JMCMAR; ISSN: 0022-2623

PB American Chemical Society

DT Journal

LA English

AB Fluoresceinyl and rhodamyl groups have been coupled by an **amide** link to side-chain amino groups at positions 1, 6, and 8 of pseudopeptide linear vasopressin antagonists through different positions on the fluorophore, to give tetraethylrhodamyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH₂, 4-HOC6H₄(CH₂)₂CO-D-Tyr(Me)-Phe-Gln-Asn-Lys(5-carboxyfluoresceinyl)-Pro-Arg-NH₂, 4-HOC6H₄(CH₂)₂CO-D-Tyr(Me)-Phe-Gln-Asn-Lys(5- or 6-carboxytetramethylrhodamyl)-Pro-Arg-NH₂, 4-HOC6H₄(CH₂)₂CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Lys(5- or 6-carboxyfluoresceinyl)-NH₂, 4-HOC6H₄(CH₂)₂CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Lys(5-carboxytetramethylrhodamyl)-NH₂ (I), and its 6-carboxytetramethylrhodamyl analog. The closer to the C-terminus the fluorophore, the higher the affinities of the fluorescent derivs. for the human vasopressin V1a receptor transfected in CHO cells. Compd. I has a K_i of 70 pM, as detd. by competition expts. with [¹²⁵I]-4-HOC6H₄CH₂CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH₂. It showed a good selectivity for human V1a receptor vs. human oxytocin (K_i = 1.2 nM), human vasopressin V1b (K_i .apprxeq. 27 nM), and human vasopressin V2 (K_i > 5000 nM) receptor subtypes. All fluorescent analogs were antagonists as shown by the inhibition of vasopressin induced inositol phosphate accumulation. These fluorescent ligands are efficient for labeling cells expressing the human V1a receptor subtype, as shown by flow cytofluorometric expts. or fluorescence microscopy. They are also appropriate tools for structural anal. of the vasopressin receptors by fluorescence.

RE.CNT 33

RE

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 144 2

L44 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:415460 HCAPLUS
 DN 129:172720
 TI Ortho-aminobenzoic acid as a fluorescent probe for the interaction between peptides and micelles
 AU Turchiello, R. F.; Lamy-Freund, M. T.; Hirata, I. Y.; Juliano, L.; Ito, A. S.
 CS Instituto de Fisica, Universidade de Sao Paulo, Sao Paulo, CEP 05315-970, Brazil
 SO Biophys. Chem. (1998), 73(3), 217-225
 CODEN: BICIAZ; ISSN: 0301-4622
 PB Elsevier Science B.V.
 DT Journal
 LA English
 AB The ortho-aminobenzoic acid (o-Abz) has been used as a fluorescent probe in internally quenched fluorescent peptides for continuous protease assays. We investigated the fluorescent properties of the probe in order to verify if it can be used to monitor the interaction of peptides with micelles. Abz-aminoacyl-monomethyl amide (Abz-Xaa-NHCH₃, where Xaa=Arg, Phe, Leu and Glu) were synthesized. Quantum yield, spectral position, anisotropy and lifetime decay were analyzed in the presence and absence of SDS (SDS) micelles. Significant changes in the fluorescence parameters were obsd. for Abz-Arg-NHCH₃ in comparison to Abz-Glu-NHCH₃, indicating a strong electrostatic component in the compds. interaction with the neg. charged micelles. The change in fluorescence parameters, obsd. when the probe is bound to hydrophobic amino acids Abz-Phe-NHCH₃ and Abz-Leu-NHCH₃, is probably due to insertion of those compds. into micelles. Abz-NHCH₃ fluorescence is less affected by the presence of micelles, indicating that the occurrence of interaction is dependent on the properties of the aminoacid to which the fluorophore is attached. The quenching data with acrylamide confirmed these results. Titrn. curves allowed the estn. of assocn. consts. between Abz compds. and SDS, according to a single partition model. Although the results cannot be strictly applied to the titrn. with charged compds., it was verified that the assocn. const. for the isolated Abz-NHCH₃ is significantly lower than those for Abz-Phe-NHCH₃ and Abz-Leu-NHCH₃. It is concluded that the Abz group is a sensitive and convenient fluorescent probe to monitor peptide binding to amphiphilic aggregates. That conclusion is supported by measurements with the peptide Abz-Leu-Arg-Phe-NH₂.

=> d bib abs hitstr 1

L48 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 2000:719189 HCAPLUS
 DN 133:344747
 TI A combination assay for simultaneous assessment of multiple signaling pathways
 AU Goetz, A. S.; Liacos, J.; Yingling, J.; Ignar, D. M.
 CS Department of Receptor Biochemistry, Glaxo Wellcome Research and Development, Research Triangle Park, NC, 27709, USA
 SO J. Pharmacol. Toxicol. Methods (1999), 42(4), 225-235
 CODEN: JPTMEZ; ISSN: 1056-8719
 PB Elsevier Science Inc.
 DT Journal
 LA English
 AB The authors have developed an assay in which modulation of two or more signaling pathways can be assessed concurrently by combining reporter gene systems with fluorescent probe technol. The validation of this method was achieved by indirect anal. of adenylyl cyclase activation with the use of a cAMP response element (CRE)-luciferase reporter system in combination with the measurement of calcium mobilization by Calcium Green-1 AM fluorescence on a fluorescent imaging plate reader. To demonstrate the utility of the method in studying the pharmacol. of receptors that couple to more than one G protein, Chinese hamster ovary (CHO) cells, which stably expressed both the CRE-luciferase reporter gene and the human pituitary adenylyl cyclase-activating peptide (PACAP) receptor, were treated with PACAP 1-27 and 1-38. Calcium mobilization and the induction of adenylyl cyclase activity in response to each concn. of peptide were assessed in individuals wells. This assay may also be used to screen for ligands of two or more unrelated receptors simultaneously without compromising the assessment of either signaling pathway. To illustrate this point, Rat-1 fibroblasts, which expressed human .alpha.1A receptors, were cocultured with CRE-luciferase CHO cells, which expressed human GLP-1 receptors. Calcium mobilization elicited by phenylephrine agonism of the .alpha.1A receptor was assessed in the same assay as GLP-1-induced activation of adenylyl cyclase. The pEC50 for each agonist was similar to that obsd. when the cell lines were not cocultured. The no. of different receptors that can be screened per well is limited only by the ability to distinguish different reporter gene signals and fluorescent indicators.

RE.CNT 23

RE

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- (2) Bevan, N; Neuroreport 1998, V9, P2703 HCAPLUS
- (4) Cheng, Y; Biochem Pharmacol 1973, V22, P3099 HCAPLUS
- (5) Ciccarelli, E; Eur J Pharmacol 1995, V288, P259 HCAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 2

L48 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 2000:658520 HCAPLUS
 DN 133:251273
 TI Metal-lipid molecules
 IN Hainfeld, James F.; Furuya, Frederic R.; Powell, Richard D.; Joshi, Vishwas N.; Gutierrez, Edmund
 PA Nanoprobe, Inc., USA
 SO U.S., 20 pp., Cont.-in-part of U.S. 5,728,590.
 CODEN: USXXAM
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6121425	A	20000919	US 1998-39601	19980316
	US 5521289	A	19960528	US 1994-282929	19940729 <--
	US 5728590	A	19980317	US 1996-652007	19960523 <--
PRAI	US 1994-282929	A2	19940729		
	US 1996-652007	A2	19960523		

AB Disclosed are novel metal-lipid mols. for **conjugating** antibody (or protein or nucleic acid) for diagnostic and therapeutic uses. The metal-lipid mols. comprise a cluster or colloid of atoms of Au, Ag, Pt, Pd, or combinations thereof, an org. group **covalently attached** to the metal atoms, and a lipid moiety. In a preferred embodiment, the metal cluster has about 50-70 gold atoms and a diam. of about 1.4 nm and the lipid moiety is dipalmitoyl phosphatidyl ethanolamine.

RE.CNT 19

RE

(1) Anon; GB 1214552 1970 HCAPLUS
 (6) Brust, M; J Chem Soc, Chem Comm 1994, P801 HCAPLUS
 (7) Danielmeyer; US 4124524 1978 HCAPLUS
 (14) Klainer; US 5116759 1992 HCAPLUS
 (15) Leuving; US 4313734 1982 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 3

L48 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1999:365078 HCAPLUS
 DN 131:211255
 TI Application of donor-donor energy migration (DDEM) for examining protein structure and function
 AU Bergstroem, Fredrik; Haggloef, Peter; Karolin, Jan; Ny, Tor; Johansson, Lennart B.-A.
 CS Dep. Physical Chemistry, Umea Univ., Umea, Swed.
 SO Proc. SPIE-Int. Soc. Opt. Eng. (1999), 3602(Advances in Fluorescence Sensing Technology IV), 250-255
 CODEN: PSISDG; ISSN: 0277-786X
 PB SPIE-The International Society for Optical Engineering
 DT Journal
 LA English
 AB Donor-Donor Energy Migration (DDEM) and fluorescence anisotropy expts. can be utilized as a versatile tool for examg. protein structure and function. For this, pairs of identical fluorescent probes (D) are **attached** to unique residues created by means of site specific mutagenesis. Present work illustrates the applicability of the method on the latent form of Plasminogen Activator Inhibitor-1 (PAI-1). Different DD-pairs of mutated PAI-1 were prepd. and studied, namely; V106C-H185C, H185C-M266C and M266C-V106C. The Cys residues were labeled with a sulfhydryl specific deriv. of BODIPY [N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-yl)methyl iodo-acetamide]. To det. the rate of DDEM within such a pair, intramol. order and dynamics must be considered. For anal. of data, addnl. information was obtained from expts. with the corresponding D-labeled single Cys mutants, i.e., V106C, H185C and M266C. The stability of values detd. was tested by generating and re-analyzing synthetic data. The intramol. distances obtained agree, reasonably well, with those detd. from the x-ray structure of latent PAI-1.

RE.CNT 11
 RE
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 (5) Johansson, L; J Chem Soc Faraday Trans 1996, V92, P1563 HCAPLUS
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 (7) Karolin, J; J Am Chem Soc 1994, V116, P7801 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 4

L48 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1999:56154 HCAPLUS
 DN 130:264230
 TI Live confocal analysis with fluorescently labeled proteins
 AU Francis-Lang, Helen; Minden, Jonathan; Sullivan, William; Oegema, Karen
 CS Department of Biology, University of California, Santa Cruz, CA, USA
 SO Methods Mol. Biol. (Totowa, N. J.) (1999), 122(Confocal
 Microscopy Methods and Protocols), 223-239
 CODEN: MMBIED; ISSN: 1064-3745
 PB Humana Press Inc.
 DT Journal
 LA English
 AB A protocol for directly **attaching** fluorescent probes to
 antibodies for live anal. and the application of this procedure more
 generally to purified and bacterially overexpressed proteins was
 described. General principles of microinjecting fluorescently labeled
 proteins, using Drosophila embryos as a specific example was presented as
 well. This technique was successfully applied in Drosophila and other
 model organisms.
 RE.CNT 24
 RE
 (2) Debec, A; J Cell Biol 1996, V134, P103 HCAPLUS
 (3) Fogarty, P; Development 1994, V120, P2131 HCAPLUS
 (6) Hird, S; Development 1996, V122, P1303 HCAPLUS
 (7) Hird, S; J Cell Sci 1996, V109, P525 HCAPLUS
 (8) Hyman, A; Methods Enzymol 1991, V196, P478 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 5

L48 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:793060 HCAPLUS
 DN 130:57170
 TI Liposomal **conjugated** peptide nucleic acids having enhanced cellular uptake
 IN Nielsen, Peter E.; Knudsen, Helle
 PA Isis Pharmaceuticals, Inc., USA
 SO PCT Int. Appl., 60 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9853801	A1	19981203	WO 1998-US10804	19980528 <--
	W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
	AU 9876021	A1	19981230	AU 1998-76021	19980528 <--
	EP 1003480	A1	20000531	EP 1998-923819	19980528
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
	JP 2001501975	T2	20010213	JP 1999-500871	19980528
PRAI	US 1997-864765	A	19970528		
	WO 1998-US10804	W	19980528		
OS	MARPAT 130:57170				
AB	Peptide nucleic acids conjugated to lipophilic groups and incorporated into liposomes exhibit enhanced cellular uptake and distribution. Cellular uptake and distribution of peptide nucleic acids also increases with the introduction of an amino acid side chain into the backbone of peptide nucleic acids. Methods of modulating cellular uptake and methods for treating animals are provided. The peptide nucleic acids of the invention comprise naturally-occurring nucleobases and non-naturally-occurring nucleobases attached to a polyamide backbone.				

RE.CNT 3

RE

- (1) Anon; Pierce ImmunoTechnology Catalog and Handbook 1992, PE65
- (2) Lansdorp; WO 9714026 A2 1997 HCAPLUS
- (3) Nielsen; US 5539082 A 1996 HCAPLUS

=> d bib abs hitstr 6

L48 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:725811 HCAPLUS
 DN 130:1900
 TI Fluorescent speckle microscopy, a method to visualize the dynamics of protein assemblies in living cells
 AU Waterman-Storer, Clare M.; Desai, Arshad; Bulinski, J. Chloe; Salmon, E. D.
 CS Department of Biology, University of North Carolina, Chapel Hill, NC, 27599-3280, USA
 SO Curr. Biol. (1998), 8(22), 1227-1230
 CODEN: CUBLE2; ISSN: 0960-9822
 PB Current Biology Publications
 DT Journal
 LA English
 AB Fluorescence microscopic visualization of fluorophore-conjugated proteins that have been microinjected or expressed in living cells and have incorporated into cellular structures has yielded much information about protein localization and dynamics. This approach has, however, been limited by high background fluorescence and the difficulty of detecting movement of fluorescent structures because of uniform labeling. These problems have been partially alleviated by the use of more cumbersome methods such as three-dimensional confocal microscopy, laser photobleaching and photoactivation of fluorescence. We report here a method called fluorescent speckle microscopy (FSM) that uses a very low concn. of fluorescent subunits, conventional wide-field fluorescence light microscopy and digital imaging with a low-noise, cooled charged coupled device (CCD) camera. A unique feature of this method is that it reveals the assembly dynamics, movement and turnover of protein assemblies throughout the image field of view at diffraction-limited resolu. We found that FSM also significantly reduces out-of-focus fluorescence and greatly improves visibility of fluorescently labeled structures and their dynamics in thick regions of living cells. Our initial applications include the measurement of microtubule movements in mitotic spindles and actin retrograde flow in migrating cells.

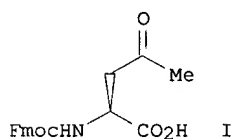
RE.CNT 15

RE

- (2) Bulinski, J; J Cell Sci 1994, V107, P2839 HCAPLUS
 - (3) Desai, A; J Cell Biol 1998, V141, P703 HCAPLUS
 - (4) Hyman, A; Meth Enzymol 1991, V196, P478 HCAPLUS
 - (5) Parsons, S; Cell Motil Cytoskeleton 1997, V36, P1 HCAPLUS
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- ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 7

L48 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:625026 HCAPLUS
 DN 129:302877
 TI Direct incorporation of unprotected ketone groups into peptides during solid-phase synthesis: application to the one-step **modification** of peptides with two different biophysical probes for FRET
 AU Marcaurelle, Lisa A.; Bertozzi, Carolyn R.
 CS Dep. Chem., Univ. California, Berkeley, CA, 94720, USA
 SO Tetrahedron Lett. (1998), 39(40), 7279-7282
 CODEN: TELEAY; ISSN: 0040-4039
 PB Elsevier Science Ltd.
 DT Journal
 LA English
 GI



AB Protected (2S)-aminolevulinic acid I (Fmoc = 9-fluorenylmethoxycarbonyl), bearing an unprotected ketone group, was incorporated into a synthetic peptide using std. Fmoc-based solid-phase methods. The ketone group remained unharmed during the synthesis and provided a uniquely reactive functional group for **covalent modification** of the peptide. The ketone and a cysteine sulfhydryl group elsewhere in the peptide were reacted simultaneously with two different biophys. probes, enabling the site-specific installation of a donor and acceptor pair for fluorescence resonance energy transfer (FRET) in one step without the need for differential side chain protection.

=> d bib abs hitstr 8

L48 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:572338 HCAPLUS
 DN 129:186415
 TI Detection of targets with green fluorescent protein and fluorescent
 variants thereof
 IN Plaia, Todd W.
 PA Oncor, Inc., USA
 SO PCT Int. Appl., 32 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9836099	A1	19980820	WO 1998-US3147	19980218 <--
W:			AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM	
RW:			GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG	
AU 9866588	A1	19980908	AU 1998-66588	19980218 <--
EP 1000172	A1	20000517	EP 1998-908588	19980218
R:			AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI	
PRAI US 1997-38623	P	19970218		
WO 1998-US3147	W	19980218		

AB A labeled marker for detection of a target is described which includes a label selected from the group consisting of green fluorescent protein and a fluorescent variant thereof, and a ligand configured to bind to the target. The ligand includes any mol. or combination of mols. which have an affinity for another substance. For example, the ligand can be selected from the group consisting of nucleic acid probe, antibody, hapten conjugate, biotin, avidin and streptavidin. A method for detecting a target is also described which includes providing a primary ligand configured to bind to the target and providing a secondary ligand configured to bind to the primary ligand. Techniques such as fluorescent microscopy are used to visualize the labeled marker.

=> d bib abs hitstr 9

L48 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2001 ACS

AN 1998:496687 HCAPLUS

DN 129:227435

TI TNP-ATP and TNP-ADP as probes of the nucleotide binding site of CheA, the histidine protein kinase in the chemotaxis signal transduction pathway of *Escherichia coli*

AU Stewart, Richard C.; VanBruggen, Ricalee; Ellefson, Dolph D.; Wolfe, Alan J.

CS Department of Cell Biology Molecular Genetics, University of Maryland, College Park, MD, 20742, USA

SO Biochemistry (1998), 37(35), 12269-12279

CODEN: BICHAU; ISSN: 0006-2960

PB American Chemical Society

DT Journal

LA English

AB The interaction of CheA with ATP has important consequences in the chemotaxis signal transduction pathway of *Escherichia coli*. This interaction results in autophosphorylation of CheA, a histidine protein kinase. Autophosphorylation of CheA sets in motion a chain of biochemical events that enables the chemotaxis receptor proteins to communicate with the flagellar motors. As a result of this communication, CheA allows the receptors to control the cell swimming pattern in response to gradients of attractant and repellent chemicals. To probe CheA interactions with ATP, we investigated the interaction of CheA with the fluorescent nucleotide analogs TNP-ATP [2'-(3')-O-(2,4,6-trinitrophenyl)ATP] and TNP-ADP. Spectroscopic studies indicated that CheA bound TNP-ATP and TNP-ADP with high affinity (micromolar K_d values) and caused a marked enhancement of the fluorescence of the TNP moiety of these modified nucleotides. Anal. of titration experiments indicated a binding stoichiometry of two mols. of TNP-ATP (TNP-ADP) per CheA dimer and suggested that the two binding sites on the CheA dimer operate independently. Binding of TNP-ATP to CheA was inhibited by ATP, and anal. of this inhibition indicated that the CheA dimer binds 2 mols. of ATP. Competition experiments also indicated that CheA binds TNP-ATP considerably more tightly than it binds unmodified ATP. Binding of TNP-ADP to CheA was inhibited by ADP in a similar manner. TNP-ATP was not a substrate for CheA and served as a potent inhibitor of CheA autophosphorylation (K_i < 1 μM). The glycine-rich regions (G1 and G2) of CheA and other histidine protein kinases have been presumed to play important roles in ATP binding and/or catalysis of CheA autophosphorylation, although few experimental tests of these functional assignments have been made. Here, we demonstrate that a CheA mutant protein with Gly₄₇₀→Ala substitutions in G1 and G2 has a markedly reduced affinity for ATP and ADP, as measured by Hummel-Dreyer chromatography. This mutant protein also bound TNP-ATP and TNP-ADP very poorly and had no detectable autokinase activity. Surprisingly, a distinct single-site substitution in G2 (Gly₄₇₀→Lys) had no observable effect on the affinity of CheA for ATP and ADP, despite the fact that it rendered CheA completely inactive as an autokinase. This mutant protein also bound TNP-ATP and TNP-ADP with affinities and stoichiometries that were indistinguishable from those observed with wild-type CheA. These results provide some preliminary insight into the possible functional roles of G1 and G2, and they suggest that TNP-nucleotides are useful tools for exploring the effects of additional mutations on the active site of CheA.

=> d bib abs hitstr 10

L48 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2001 ACS

AN 1998:425671 HCAPLUS

DN 129:158836

TI Strategies for positioning fluorescent probes and crosslinkers on formyl peptide ligands

AU Vilven, Janeen C.; Domalewski, Mark; Prossnitz, Eric R.; Ye, Richard D.; Muthukumaraswamy, Natesa; Harris, Robert B.; Freer, Richard J.; Sklar, Larry A.

CS Cancer Center and Pathology, Univ. New Mexico Health Sciences Cent., Albuquerque, NM, USA

SO J. Recept. Signal Transduction Res. (1998), 18(2 & 3), 187-221

CODEN: JRETET; ISSN: 1079-9893

PB Marcel Dekker, Inc.

DT Journal

LA English

AB Chemoattractant receptors represent a major subset of the G-protein coupled receptor (GPCR) family. One of the best characterized, the N-formyl peptide receptor (FPR), participates in host defense responses of neutrophils. The features of the ligand which regulate its interaction with the FPR are well-known. By manipulating these features we have developed new ligands to probe structural and mechanistic aspects of the peptide-receptor interaction. Three ligand groups have been developed: (1) ligands containing a Lys residue located in positions 2 through 7 that can be **conjugated** to FITC (N-formyl-Met1-Lys2-Phe3-Phe4, N-formyl-Met1-Leu2-Lys3-Phe4, N-formyl-Met1-Leu2-Phe3-Lys4, N-formyl-Met1-Leu2-Phe3-Phe4-Lys5, N-formyl-nLeu1-Leu2-Phe3-nLeu4-Tyr5-Lys6 and N-formyl-Met1-Leu2-Phe3-Phe4-Gly5-Gly6-Lys7); (2) fluorescent pentapeptide ligands (N-formyl-Met-X-Phe-Phe-Lys(FITC) where X = Leu, Ala, Val or Gly); and (3) small crosslinking ligands where the photoaffinity crosslinker 4-azidosalicylic acid (ASA) was **conjugated** to Lys in positions 3 and 4 and p-benzoyl-phenylalanine (Bpa) was located in position 2 in N-formyl-Met1-Bpa2-Phe3-Tyr4. The peptides were characterized according to activity and affinity in human neutrophils and cell lines transfected with FPR. All of the peptides were agonists, with parallel affinity and activity. In the first group, the peptide activity decreases as Lys is placed closer to the N-formyl group and the activity is improved by 1-3 orders of magnitude by **conjugation** with FITC. In the second group, the dissociation rate of the peptide from the receptor increases as position 2 is replaced by aliph. amino acids with smaller alkyl groups. In the third group, crosslinking ligands remain biologically active, display nM affinity and **covalently** label the FPR.

=> d bib abs hitstr 11

L48 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:296507 HCAPLUS
 DN 129:52466
 TI Visualization of dynamic trafficking of a protein kinase C .beta.II/green fluorescent protein **conjugate** reveals differences in G protein-**coupled** receptor activation and desensitization
 AU Feng, Xiao; Zhang, Jie; Barak, Larry S.; Meyer, Tobias; Caron, Marc G.; Hannun, Yusuf A.
 CS Department of Cell Biology, Duke University Medical Center, Durham, NC, 27710, USA
 SO J. Biol. Chem. (1998), 273(17), 10755-10762
 CODEN: JBCHA3; ISSN: 0021-9258
 PB American Society for Biochemistry and Molecular Biology
 DT Journal
 LA English
 AB Protein kinase C (PKC) **links** various extracellular signals to intracellular responses and is activated by diverse intracellular factors including diacylglycerol, Ca²⁺, and arachidonic acid. In this study, using a fully functional green fluorescent protein-**conjugated** PKC.beta.II (GFP-PKC.beta.II), we demonstrate a novel approach to study the dynamic redistribution of PKC in live cells in response to G protein-**coupled** receptor activation. Agonist-induced PKC translocation was rapid, transient, and selectively mediated by the activation of Gq.alpha.- but not Gs.alpha.- or Gi.alpha.-**coupled** receptors. Interestingly, although the stimuli were continuously present, only one brief peak of PKC membrane translocation was obsd., consistent with rapid desensitization of the signaling pathway. Moreover, when GFP-PKC.beta.II was used to examine cross-talk between two Gq.alpha.-**coupled** receptors, angiotensin II type 1A receptor (AT1AR) and endothelin A receptor (ETAR), activation of ETARs resulted in a subsequent loss of AT1AR responsiveness, whereas stimulation of AT1ARs did not cause desensitization of the ETAR signaling. The development of GFP-PKC.beta.II has allowed not only the real time visualization of the dynamic PKC trafficking in live cells in response to physiol. stimuli but has also provided a direct and sensitive means in the assessment of activation and desensitization of receptors implicated in the phospholipase C signaling pathway.

=> d bib abs hitstr 12

L48 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1998:159538 HCAPLUS
 DN 128:280469
 TI Detection and quantitation of heterotrimeric G proteins by fluorescence resonance energy transfer
 AU Remmers, Ann E.
 CS Department of Pharmacology, The University of Michigan, Ann Arbor, MI, 48109-0632, USA
 SO Anal. Biochem. (1998), 257(1), 89-94
 CODEN: ANBCA2; ISSN: 0003-2697
 PB Academic Press
 DT Journal
 LA English
 AB N-Methyl-3'-O-anthranoyl (mant) guanine nucleotide analogs are useful environmentally sensitive fluorescent probes for detection of heterotrimeric guanine nucleotide binding proteins. The mant deriv. of GTP.gamma.S, mGTP.gamma.S, is synthesized and purified by modification of a method initially described by T. Hiratsuka (1983). The binding affinity of mGTP.gamma.S for G proteins Gi and Go is comparable to that of GTP.gamma.S. The rate of binding is detd. by the dissochn. rate of the endogenously bound GDP. The large fluorescence increase obsd. upon mGTP.gamma.S binding to G protein is due, in part, to resonance energy transfer from tryptophans in the G protein to the mant guanine nucleotide. The magnitude of the fluorescence increase is dependent upon the concn. of G protein. Therefore, mGTP.gamma.S binding can be used to quantitate and locate G proteins during the protein purifn. process. This method is rapid compared to the [35S]GTP.gamma.S binding assay in that (i) the bound ligand does not need to be sepd. from the free ligand thus avoiding vacuum filtration and (ii) the time required to measure fluorescence in each sample is less than that required for scintillation counting. In addn., the use of radioactivity can be avoided. Thus, the mGTP.gamma.S binding assay for the detection of Gi and Go represents a rapid, reliable alternative to assays based on radiolabeled GTP.gamma.S binding or ADP-ribosylation with pertussis toxin.

=> d bib abs hitstr 13

L48 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:772086 HCAPLUS
DN 128:112486
TI The phytofluors: a new class of fluorescent protein probes
AU Murphy, John T.; Lagarias, J. Clark
CS Section of Molecular and Cellular Biology, University of California,
Davis, CA, 9561 6, USA
SO Curr. Biol. (1997), 7(11), 870-876
CODEN: CUBLE2; ISSN: 0960-9822
PB Current Biology Ltd.
DT Journal
LA English
AB Biol. compatible fluorescent protein probes, particularly the
self-assembling green fluorescent protein (GFP) from the jellyfish
Aequorea victoria, have revolutionized research in cell, mol. and
developmental biol. because they allow visualization of biochem. events in
living cells. Addnl. fluorescent proteins that could be reconstituted in
vivo while extending the useful wavelength range towards the orange and
red regions of the light spectrum would increase the range of applications
currently available with fluorescent protein probes. Intensely orange
fluorescent adducts, which we designate phytofluors, are spontaneously
formed upon incubation of recombinant plant phytochrome apoproteins with
phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin
chromophore. Phytofluors have large molar absorption coeffs.,
fluorescence quantum yields greater than 0.7, excellent photostability,
stability over a wide range of pH, and can be reconstituted in living
plant cells. The phytofluors constitute a new class of fluorophore that
can potentially be produced upon bilin uptake by any living cell
expressing an apophytochrome cDNA. Mutagenesis of the phytochrome
apoprotein and/or alteration of the linear tetrapyrrole precursor by chem.
synthesis are expected to afford new phytofluors with fluorescence
excitation and emission spectra spanning the visible to near-IR light
spectrum.

=> d bib abs hitstr 14

L48 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2001 ACS

AN 1997:332770 HCAPLUS

DN 127:62698

TI Detection in living cells of Ca^{2+} -dependent changes in the fluorescence emission of an indicator composed of two green fluorescent protein variants **linked** by a calmodulin-binding sequence. A new class of fluorescent indicators

AU Romoser, Valerie A.; Hinkle, Patricia M.; Persechini, Anthony

CS Department Pharmacology Physiology, University Rochester Medical Center, Rochester, NY, 14642, USA

SO J. Biol. Chem. (1997), 272(20), 13270-13274

CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB We have designed a novel fluorescent indicator composed of two green fluorescent protein variants joined by the calmodulin-binding domain from smooth muscle myosin light chain kinase. When $(\text{Ca}^{2+})_4$ -calmodulin is bound to the indicator ($K_d = 0.4 \text{ nM}$), fluorescence resonance energy transfer between the two fluorophores is attenuated; the ratio of the fluorescence intensity measured at 505 nm to the intensity measured at 440 nm decreases 6-fold. Images of microinjected living cells demonstrate that emission ratios can be used to monitor spatio-temporal changes in the fluorescence of the indicator. Changes in indicator fluorescence in these cells are **coupled** with no discernible lag ($<1 \text{ s}$) to changes in the cytosolic free Ca^{2+} ion concn., ranging from below 50 nM to $\text{apprx. } 1 \text{ } \mu\text{M}$. This observation suggests that the activity of a calmodulin target with a typical 1 nM affinity for $(\text{Ca}^{2+})_4$ -calmodulin is responsive to changes in the intracellular Ca^{2+} concn. over the physiol. range. It is likely that the indicator we describe can be **modified** to detect the levels of ligands and proteins in the cell other than calmodulin.

=> d bib abs hitstr 15

L48 ANSWER 15 OF 15 HCAPLUS COPYRIGHT 2001 ACS
 AN 1997:114489 HCAPLUS
 DN 126:222460
 TI Fluorescence spectroscopy as a tool to investigate protein interactions
 AU Brown, Martha P.; Royer, Catherine
 CS School of Pharmacy, University of Wisconsin-Madison, Madison, WI, 53706, USA
 SO Curr. Opin. Biotechnol. (1997), 8(1), 45-49
 CODEN: CUOBE3; ISSN: 0958-1669
 PB Current Biology
 DT Journal
 LA English
 AB Recent advances in the use of fluorescence spectroscopy to study protein interactions have primarily involved combinations of classic fluorescence techniques, novel probe and **coupling** chemistries, and advances in laser excitation and detection capabilities. For example, new **coupling** strategies for fluorescent probes have allowed the first detn. of the .DELTA.G* describing the insertion of a protein into a membrane. Fluorescently labeled oligonucleotides with specific protein-binding sequences have been used to study both protein-DNA assocns. and oligonucleotide hybridization using anisotropy changes. The first kinetic data describing a DNA-protein binding event was collected with stopped-flow fluorescence instrumentation. Combining scanning fluctuation correlation spectroscopy with a two-photon excitation source improved this technique so that it may now be used to study protein self-assocns.

=> d bib abs 1

L51 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1997:78252 HCAPLUS
 DN 126:180823
 TI Design, synthesis, and in vitro evaluation of cytotoxic analogs of bombesin-like peptides containing doxorubicin or its intensely potent derivative, 2-pyrrolinodoxorubicin
 AU Nagy, Attila; Armatis, Patricia; Cai, Ren-Zhi; Szepeshazi, Karoly; Halmos, Gabor; Schally, Andrew W.
 CS Endocrine, Polypeptide Cancer Institute, Tulane University School of Medicine, New Orleans, LA, 70146, USA
 SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(2), 652-656
 CODEN: PNASA6; ISSN: 0027-8424
 PB National Academy of Sciences
 DT Journal
 LA English
 AB Five peptide fragments, based on the C-terminal sequence of **bombesin** (BN)-(6-14) or BN-(7-14), were selected as carriers for radicals doxorubicin (DOX) and 2-pyrrolino-DOX to create hybrid cytotoxic analogs. All these compds. had a reduced **peptide bond** (CH2-NH or CH2-N) between positions 13 (Phe or Leu) and 14 (Phe, Leu, or Tac) (Tac = thiazolidine-4-carboxylic acid). Three pseudonona-peptide carriers contained N-terminal D-Phe or D-Tpi at position 6 (Tpi = 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid). Two pseudoocta-peptides had Gln7 at the N terminus. The conjugation of N-(9-**fluorenylmethoxycarbonyl**)doxorubicin (N-Fmoc-DOX)-14-O-hemiglutarate to the peptide carriers at the N terminus resulted in cytotoxic hybrids of BN-like peptides contg. DOX. These hybrids could then be converted to analogs with 2-pyrrolino-DOX by a reaction with 4-iodobutyraldehyde. The ability of the carriers and the conjugates to inhibit the binding of 125I-labeled [Tyr4]BN to receptors for BN/**gastrin releasing peptide (GRP)** on Swiss 3T3 cells was detd. Cytotoxic conjugates of pseudoocta-peptide carrier analogs displayed the highest binding affinity (KD .apprxeq.1 nM). The cytotoxic BN analogs and their corresponding cytotoxic radicals exerted similar inhibitory effects on the in vitro growth of CFPAC-1 human pancreatic cancer, DMS-53 human lung cancer, PC-3 human prostate cancer, and MKN-45 human gastric cancer cell lines that have receptors for BN/**GRP**. In DMS-53 cells, the activity of 2-pyrrolino-DOX and its conjugates was .apprxeq.2500 times higher than that of DOX and its hybrids. These highly potent cytotoxic analogs of BN have been designed as targeted anti-tumor agents for the treatment of various cancers that possess receptors for BN/**GRP**.

=> d bib abs 2

L51 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1995:922486 HCAPLUS
 DN 124:117903
 TI Novel Carboxylic Acid and Carboxamide Protective Groups Based on the
 Exceptional Stabilization of the Cyclopropylmethyl Cation
 AU Carpino, Louis A.; Chao, Hann Guang; Ghassemi, Shahnaz; Mansour, E. M. E.;
 Riemer, Christoph; Warrass, Ralf; Sadat-Aalae, Dean; Truran, George A.;
 Imazumi, Hideko; et al.
 CS Wenschuh, Holger, Department of Chemistry, University of Massachusetts,
 Amherst, MA, 01003-4510, USA; Beyermann, Michael; Bienert, Michael;
 Shroff, Hitesh; Albericio, Fernando; Triolo, Salvatore A.; Sole, Nuria A.;
 Kates, Steven A.
 SO J. Org. Chem. (1995), 60(24), 7718-19
 CODEN: JOCEAH; ISSN: 0022-3263
 DT Journal
 LA English
 OS CASREACT 124:117903
 AB The secondary dicyclopropylmethyl (Dcpm) group has been shown to be useful
 for protection of the carboxylic acid function of peptides in cases where
 selective deblocking in the presence of tert-Bu based or trityl side chain
 protection is desired. Examples involved synthesis of protected
 tripeptide Fmoc-Gly-Asp(OCMe₃)-Ala-OH (Fmoc = 9-
fluorenylmethoxycarbonyl) and hexapeptide Fmoc-Ile-Thr(CMe₃)-
 Arg(Pmc)-Gln(CPh₃)-Arg(Pmc)-Tyr(CMe₃)-OH (Pmc = 2,2,5,7,8-
 pentamethylchroman-6-sulfonyl), both of which were assembled by soln.
 methods. Comparable **amide** protection was possible via the
 dimethylcyclopropylmethyl (Dmcp) residue which could be deblocked along
 with tert-Bu-based side chain protecting groups by CF₃CO₂H. The new Dmcp
 group was used for protection of Asn and Gln during the solid phase
 syntheses of a no. of peptides contg. these two amino acids [acyl carrier
 protein(65-74), **bombesin**, etc.]. Dmcp protection of the
 C-terminal **amide** function allowed development of a novel direct
 synthesis of peptide **amides** by the rapid soln. approach. No
 evidence was obtained for retention of isomeric Dcpm or Dmcp residues
 during the final deblocking step thus showing that no unproductive ring
 expansion rearrangement occurred in the presence of acid.

=> d bib abs 3

L51 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1994:474075 HCAPLUS
 DN 121:74075
 TI Potent pseudopeptide bombesin-like agonists and antagonists: correlation of ordered conformation of bombesin analogs to receptor activity
 AU Edwards, J. Vincent; McLean, Larry R.; Wade, Arlene C.; Eaton, Scott R.; Cashman, Elizabeth A.; Hagaman, Karen A.; Fanger, Bradford O.
 CS Marion Merrell Dow Res. Inst., Cincinnati, OH, USA
 SO Int. J. Pept. Protein Res. (1994), 43(4), 374-83
 CODEN: IJPPC3; ISSN: 0367-8377
 DT Journal
 LA English
 AB **Bombesin**-like pseudopeptides have been synthesized, and certain physicochem. properties and biol. activities have been examd. **Bombesin** and the related peptide litorin were modified at positions 13-14 and 8-9, resp., with .psi.[CH2S] and .psi.[CH2N(CH3)]. [Phe13.psi.[CH2S]Leu14]**bombesin** and [Phe8.psi.[CH2S]-Leu9]litorin bound to the murine pancreatic **bombesin/gastrin-releasing** peptide receptor with similar dissocn. consts. ($K_d = 3.9$ and 3.4 nM, resp.). Increased potency was achieved by oxidn. of the thiomethylene ether to two diastereomeric sulfoxides (isomer I, $K_d = 1.6$ nM and isomer II, $K_d = 0.89$ nM). Further oxidn. to the sulfone decreased potency ([Phe8.psi.[CH2SO2]Leu9]litorin, $K_d = 9.9$ nM). All five analogs were receptor antagonists as detd. by phosphatidylinositol turnover in murine pancreas. In contrast to these peptide backbone substitutions, a .psi.[CH2N(CH3)] at the 8-9 **amide** bond position resulted in an agonist. The analogs were compared with those of litorin ($K_d = 0.1$ nM) and [Leu9]litorin ($K_d = 0.17$ nM) by CD and **fluorescence** spectroscopy. The CD spectra demonstrated ordered conformation for all the peptides in TFE. Different conformations corresponding to agonist and antagonist peptides were suggested by CD. Based on the pH-dependence of the **fluorescence** spectra of the peptides in a zwitterionic detergent, two titratable groups were identified ($pK_a = 6.3$ and 8.5). The lower pK_a is found in the agonist analogs but not in the .psi.[CH2S]-contg. antagonist.

=> d bib abs 4

L51 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1990:199089 HCAPLUS
 DN 112:199089
 TI Solid-phase synthesis of bombesin by continuous flow procedure using
 Fmoc-amino acids
 AU Scolaro, B.; Gozzini, L.; Rocchi, Raniero; Di Bello, C.
 CS Biopolym. Res. Cent., Univ. Padova, Padua, 35131, Italy
 SO Int. J. Pept. Protein Res. (1989), 34(5), 423-9
 CODEN: IJPPC3; ISSN: 0367-8377
 DT Journal
 LA English
 GI

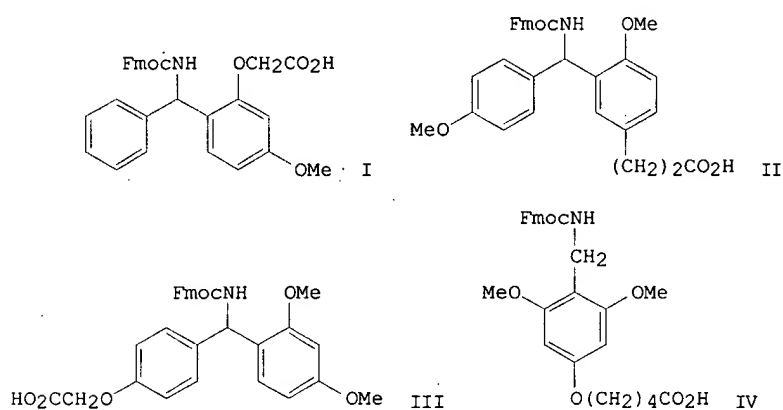
pyroGlu-Gln-Arg-Leu-Gly-Asn-Gln-

Trp-Ala-Val-Gly-His-Leu-Met-NH₂ I

AB **Bombesin** (I) has been synthesized by the continuous flow solid-phase procedure on the derivatized Kieselguhr-supported polydimethylacrylamide resin. Preformed Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) amino acid sym. anhydrides (Met, Leu, and Arg) and Fmoc amino acid active esters were used for amine acylation. The 4-methoxy-2,3,6-trimethylbenzenesulfonyl and 2,2,5,7,8-pentamethylchroman-6-sulfonyl groups have been alternatively used for masking the side chain function of Arg-3. The progress of the synthesis was monitored by different anal. methods including quant. solid-phase Edman degradn. Cleavage from the resin and simultaneous formation of the C-terminal **amide** function were achieved with a methanolic ammonia soln. yielding indistinguishable crude peptides which have been purified by HPLC and fully characterized. Preliminary pharmacol. expts. indicated that the activity of the synthetic peptides is similar to that previously measured for other synthetic **bombesins**. For comparison **bombesin** has also been prepd. by solid-phase synthesis on 4-methylbenzhydrylamine resin using the Boc chem. The results of the two strategies are discussed and compared.

=> d bib abs 5

L51 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1990:199065 HCAPLUS
 DN 112:199065
 TI A comparison of acid-labile linkage agents for the synthesis of peptide C-terminal amides
 AU Bernatowicz, Michael S.; Daniels, Scott B.; Koster, Hubert
 CS Milligen/Bioscience Div., Millipore, Burlington, MA, 01803, USA
 SO Tetrahedron Lett. (1989), 30(35), 4645-8
 CODEN: TELEAY; ISSN: 0040-4039
 DT Journal
 LA English
 GI

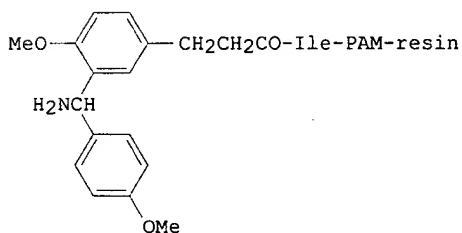
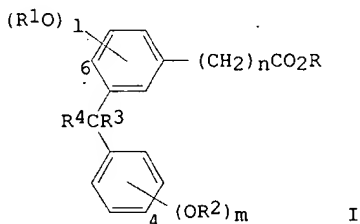


AB Substituted benzhydrylamine and benzylamine linkage agents I-IV (Fmoc = 9-fluorenylmethoxycarbonyl) useful for the solid-phase peptide synthesis of C-terminal amides were evaluated for their relative lability toward CF₃CO₂H. The two most reactive linkage agents studied were compared in the synthesis of two different peptide amides (eledoisin and neuromedin U-25) by the Fmoc protecting group strategy.

=> d bib abs 6

L51 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1990:77970 HCAPLUS
 DN 112:77970
 TI Preparation of benzhydrylamine derivatives as reagents for solid-phase synthesis of polypeptide amides
 IN Funakoshi, Susumu; Murayama, Eigo
 PA Chugai Pharmaceutical Co., Ltd., Japan
 SO Eur. Pat. Appl., 11 pp.
 CODEN: EPXXDW
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 331073	A2	19890906	EP 1989-103397	19890227
	EP 331073	A3	19901114		
	EP 331073	B1	19930728		
	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
	JP 01221358	A2	19890904	JP 1988-46710	19880229
	US 4965405	A	19901023	US 1989-316167	19890227
	AT 92039	E	19930815	AT 1989-103397	19890227
	US 5051526	A	19910924	US 1990-527806	19900524
PRAI	JP 1988-46710		19880229		
	EP 1989-103397		19890227		
	US 1989-316167		19890227		
OS	MARPAT 112:77970				
GI					

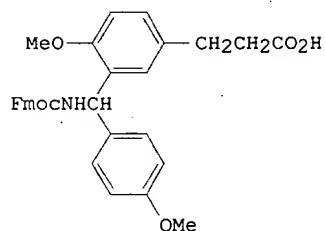


AB Benzhydrylamine derivs. (I; R = R4 = H; R1, R2 = C1-3 alkyl; 1, m = 1, 2; n = 1-4; R3 = FMOCNH; FMOC = 9-fluorenylmethyloxycarbonyl), useful as reagents for modification of an aminomethylated polystyrene resin in the solid-phase synthesis of polypeptide amides using Fmoc protective group, are prepd. via intermediates I (R3 = H2N, R4 = H; R3R4 = O). Thus, Friedel-Crafts acylation of 4-MeOC6H4CH2CH2CO2Me with p-MeOC6H4COCl in PhNO2 in the presence of AlCl3 gave I [R = Me, (R1O)1 = 6-MeO, (OR2)m = 4-OMe, R3R4 = O] which was saponified with NaOH in aq. MeOH, oxidized with H2NOH.HCl, and then reduced with Zn in AcOH to give I [R = R4 = H, (R1O)1 = 6-MeO, (OR2)m = 4-OMe, R3 = NH2]. Acylation of the latter with O-(9-fluorenylmethyloxycarbonyl)-N-hydroxysuccinimide in DMF contg. Et3N gave I [R = R4 = H, (R1O)1 = 6-MeO,

(OR2)_m = 4-OMe, R3 = FMOCNH] which was coupled to H-Ile-PAM-polystyrene resin (PAM = phenylacetamidomethyl) in DMF in the presence of DCC and 1-hydroxybenzotriazole to give a modified resin (II). Tetragastrin, neuromedin B, and arginine-vasopressin were prepd. by the solid-phase method using II and FMOC protected amino acids.

=> d bib abs 7

L51 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2001 ACS
 AN 1989:633558 HCAPLUS
 DN 111:233558
 TI Studies on peptides. CLXI. A modified benzydrylamine. A useful handle reagent for 9-fluorenylmethyloxycarbonyl based solid phase synthesis of peptide amides
 AU Funakoshi, Susumu; Murayama, Eigoro; Guo, Lili; Fujii, Nobutaka; Yajima, Haruaki
 CS Fac. Pharm. Sci., Kyoto Univ., Kyoto, 606, Japan
 SO Collect. Czech. Chem. Commun. (1988), 53(11B), 2791-800
 CODEN: CCCCAK; ISSN: 0010-0765
 DT Journal
 LA English
 OS CASREACT 111:233558
 GI



AB Dimethoxybenzhydrylamine deriv. I (Fmoc = 9-fluorenylmethyloxycarbonyl) was prepd. and used as a handle reagent for Fmoc-based solid-phase synthesis of peptide **amides** tetragastrin, **neuromedin B**, and [8-arginine]vasopressin. 1M Me3SiBr-PhSH (molar ratio 1:1) in CF3CO2H was recommended as a deprotecting reagent for releasing the peptide **amides** from the resin.